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13. ABSTRACT *(Maximum 200 words)*

The feasibility of fabricating a multi-compartment intravitreal implant for treatment of traction retinal detachment was successfully confirmed. The goal was to design a biodegradable implant capable of pulsatile release of 5-fluorouracil (5-FU) and continuous release of diclofenac. TheriForm technology was used to construct several prototypes; the walls and lids being made with biodegradable polymers of controlled composition and thickness to optimize drug release. Polymer selection was based on film degradation studies and iterative design improvements. All initial prototypes comprised one drug only, but included a placebo structure to mimic the portion that would contain the other drug in the composite commercial dosage form. This tactic simplified product development and demonstrated process capabilities to fabricate implants with therapeutic agents in separate compartments. The 5-FU-only implants successfully demonstrated pulsatile release with lag times of 0-18 hours. Ongoing research has provided several leads to increase inter-pulse time delay. For the diclofenac-only portion, the burst effect was eliminated by covering the diclofenac-containing matrix with placebo polymer layers and including 35% (w/w) sodium chloride as a representative, soluble, inert filler to build interconnecting channels. Using this strategy, a constant drug release of 80 μg/day was achieved. This research project demonstrated the potential of the TheriForm process to manufacture unique implants capable of prescriptive release of several drugs, not only for treatment of ocular diseases, but to serve as a platform for multiple applications.

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Donald C. Monkhouse 21 May 97

PI - Signature Date

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RETINAL DRUG DELIVERY SYSTEM: PHASE I FINAL REPORT

1. INTRODUCTION

1.1. Retinal drug delivery

The unique anatomy of the eye presents three major challenges for intraocular drug delivery. First, the blood-retinal barrier or cornea must be crossed for delivery to the inner eye. Second, the drug activity must be above the minimum therapeutic level in the eye but must not reach toxic levels in the rest of the body. Third, intraocular therapeutic levels of the drug must be reached at prescribed levels with minimum administration frequency to improve safety and patient compliance. Conventional methods of delivery, such as systemic, topical, and ophthalmic, cannot adequately meet these three critical parameters for most therapeutic agents.

Systemic drug delivery, such as oral or intravenous (IV) administration, falls short of all three goals mentioned above. To penetrate the blood-retinal barrier in sufficient quantities, drugs given systemically require very high doses that often lead to undesired side effects. For example, in immunocompromised patients receiving IV ganciclovir for cytomegalovirus (CMV) retinitis, 16% have dose-limiting neutropenia and 5% have dose-limiting thrombocytopenia.[1] In addition, systemic drugs usually have to be administered frequently. For example, ganciclovir has a plasma half-life of 3-4 hours and must be administered daily.[1]

Topical delivery bypasses the blood-retinal barrier and reduces systemic side effects, but the cornea must be traversed and frequent administration is necessary. Topical drops and ointments are convenient but they usually are effective for only a short period of time, and therefore must be administered frequently, which decreases patient compliance. Perfusion into the conjunctival sac is cumbersome, expensive, and uncomfortable. Contact lenses soaked in a drug solution can deliver ten times as much drug intraocularly than ophthalmic drops.[2] However, this method results in variable and imprecise bioavailability owing to differing levels of drug absorption by the lenses due to differences in materials used to make the lenses, concentration of drug in the soaking solution, and soaking time. Inserts placed in the conjunctival sac, such as the Ocusert™, need to be replaced once a week and must be checked twice a day because 20% of the users lose them without realizing it.[3] Drugs delivered topically often do not reach therapeutic levels in the posterior part of the eye, which is important in many diseases, such as age-related macular degeneration (AMD) and diabetic retinopathy.

Intraocular injection can place the drug directly into the vitreous cavity but is not recommended in most cases owing to complications, such as endophthalmitis, retinal detachment, elevated intraocular pressure, and optic atrophy. In addition, injection into the eye is not tolerated well by patients. Furthermore, only drugs that are very lipophobic, and therefore do not penetrate the eye well, are candidates for intraocular injection.

In contrast to the above, intravitreal delivery bypasses the cornea and the blood-retinal barrier and requires less drug than other modes of administration, thereby reducing systemic side effects. Intraocular systems provide long-term, localized drug delivery and significantly reduce the frequency of administration thereby increasing patient compliance. Also, efficacy is increased and at the same time, local and systemic side effects are diminished. Controlled release systems would be especially useful for chronic conditions such as proliferative diseases, glaucoma, CMV, dry eye, and inflammation. Many of these conditions require treatment with multiple drugs. For example, traction retinal detachment caused by leukocyte proliferation requires an anti-proliferative and an anti-inflammatory; and glaucoma patients are treated simultaneously with sympathomimetics, beta blockers, hyperosmotic agents, and carbonic anhydrase inhibitors. In addition, many inner eye injuries, such as laser induced retinal lesions, need an antibiotic, an anti-inflammatory agent, and an analgesic, and, recent research suggests that it might be advantageous to treat CMV retinitis with both ganciclovir and foscarnet.[4] Current intravitreal systems, such as Vitrasert™ and Oculex™, deliver one drug only. Other disadvantages are that Vitrasert is non-biodegradable and therefore must be surgically removed, and Oculex microparticles release most of the drug in an initial burst. Neither system delivers the drug in a pulsatile fashion.

1.2. Fabrication of intraocular implants using TheriForm™ process

A novel biodegradable retinal drug delivery system with the capability of delivering one or more drugs, either at the same rate or at independent rates and at predetermined release rates, would offer new opportunities for treating retinal diseases and injuries. TheriForm™ (Therics' proprietary three dimensional printing [3DP] technology) exhibits the unique capability to create a system that releases therapeutic agents in a "***prescriptive***" (precisely predetermined) manner, that is, control over time, amount and sequence, either continuously or pulsed. This system would have all of the advantages of intravitreal delivery, as well as the benefit of delivering agents at individually prescribed rates. This report describes the preliminary research conducted to develop an intravitreal implant for treatment of traction retinal detachment. A detailed description of the TheriForm™ process is provided in Appendix A.

1.3. Traction retinal detachment

Proliferative diabetic retinopathy, proliferative vitreoretinopathy, and penetrating ocular trauma are conditions that often result in traction retinal detachment. Traction retinal detachment occurs when one of these diseases or injury incites inflammation within the posterior segment of the eye, resulting in the breakdown of the blood-retinal barrier. These events stimulate wound-healing responses, which in turn, initiate the migration and proliferation of retinal cells within the posterior segment. When the cells attain a critical mass, retinal detachment occurs. This leads to further breakdown of the blood-retinal barrier and inflammation, thereby accelerating the process. Pterygium is a hot climate disease in which the proliferation of the conjunctiva over the cornea occurs. Posterior capsule opacification is a condition that occurs 15-50% of the time after extracapsular cataract extraction, and is characterized by the proliferation and migration of remnant lens epithelial cells that can form fibrosis or pearls on the posterior lens capsule. Anti-proliferative agents and anti-inflammatory drugs are used to treat all of these conditions.

1.4. Choice of drugs

For this project, 5-Fluorouracil (5-FU) and diclofenac sodium (diclofenac, DC) were chosen as the anti-proliferative and the anti-inflammatory, respectively. 5-FU is a fluorinated pyrimidine analog that is frequently used as an anti-metabolite and has been shown to inhibit fibroblast proliferation.[5,6] Diclofenac is a phenylacetic acid that has exhibited anti-inflammatory and analgesic properties in pharmacological studies.[7] It has been demonstrated that diclofenac inhibits the enzyme cyclooxygenase, which is required in the synthesis of prostaglandins. Prostaglandins are mediators of some types of intraocular inflammation.

Many therapeutic agents, such as 5-FU, can be extremely toxic; in such cases, pulsed delivery would produce the same therapeutic benefits while reducing side effects and toxic exposure. Recent studies have shown the advantages of pulse dosing of 5-FU in the topical treatment of facial actinic keratoses.[8] In addition, contemporary wisdom teaches that Multiple Drug Resistance (MDR), often encountered with anti-neoplastic agents such as 5-FU, occurs due to the P-Glycoprotein efflux mechanism. Here, following continuous exposure to low levels of drug, the cell adapts to pump out the intrusive agent even faster than the rate at which it enters. Hence, the cell is never exposed to therapeutic levels and thus becomes resistant. The principal hypothesis of this proposal is that through pulsing, the P-Glycoprotein mechanism or the like (such as cytochrome P450 3A) can be effectively circumvented. Therefore, it is desired that 5-FU be released from the intravitreal implant in a pulsatile manner. On the other hand, the anti-inflammatory agent does not apparently suffer from this defense tactic by the cell, and so the plan is to deliver this drug at a continuous rate.

1.5. Technical objectives

The overall goal of this project is to demonstrate the utility and versatility of TheriForm™ technology in the development of retinal controlled delivery systems that cannot be achieved by conventional methods. A biodegradable TheriForm™ system of 5-FU and diclofenac would provide a unique treatment option for proliferative retinal conditions. The objective of Phase I studies was to create and test prototypes of biodegradable, intravitreal prescriptive systems that release 5-FU in a pulsed manner and release diclofenac at a continuous rate. Studies in the treatment of actinic keratoses have shown that pulsed delivery maintains therapeutic benefits and decreases toxic side effects as well as cost.[8] The specific aims of the Phase I studies are listed below:

1. Develop analytical methods for assay of 5-FU and diclofenac to determine drug content uniformity and drug release rates from implant prototypes.

2. Develop a monotherapy, erodible, polymer implant prototype for pulsatile delivery of 5-FU, preferably four pulses at four day intervals.

3. Develop a monotherapy, erodible, polymer implant prototype for continuous delivery of diclofenac, with a target release rate of 80 μg/day.

Once the two implants have been optimized independently to achieve the desired, prescriptive release rates of 5-FU and diclofenac, they are intended to be combined into one device, which should be easily accomplished by TheriForm™ fabrication during a single manufacturing process. The task of optimizing drug release from the two implants and combining them into one device, along with stability testing for shelf-life determination and animal testing for in-vivo pharmacokinetic evaluation will be conducted in Phase II of the project.

1.6. Implant design

A schematic of the proposed design of the implant is shown in Figure 1. The prototypes were to be constructed in a laminated fashion. The initial design comprised of two components. The top part consisted of four separate chambers, each containing a single pulse dose of 5-FU. The walls of the chambers were to be fabricated using polymers that degrade very slowly. Each of these chambers were to be covered with "lids" that degrade at different rates based on their composition and/or thickness. The idea was to provide lag times so that 5-FU is released from each of the chambers at 4 day intervals, before it has a chance to release through the side walls. The bottom portion was intended to comprise a polymer matrix containing diclofenac. The mechanism of diclofenac release from the matrix could be due to erosion of the polymer, diffusion of the drug through the polymer matrix, or a combination of both, depending on the composition. The proposed dimensions of the prototype delivery system were selected to be in the same range as other structures designed as intravitreal implants.[9]

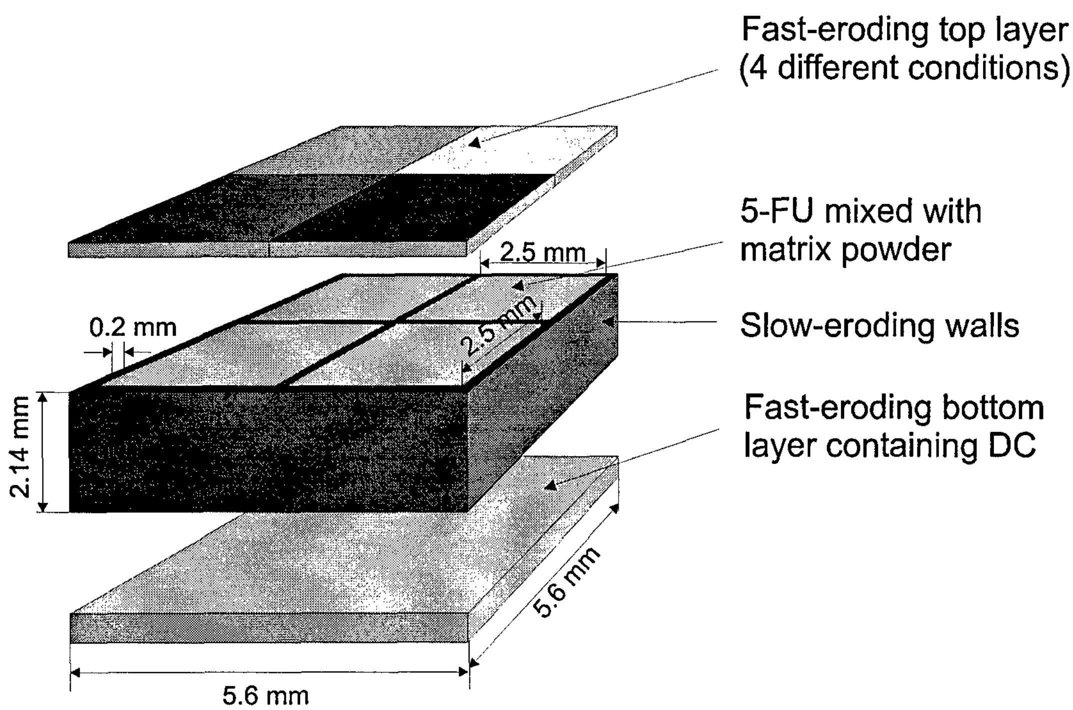
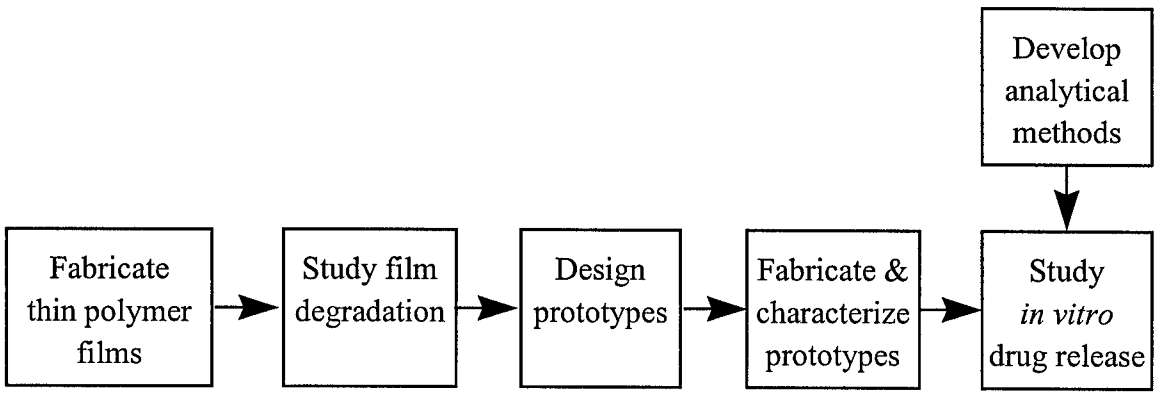


Figure 1. Design of intravitreal implant for pulsatile delivery of 5-FU and continuous release of diclofenac.

1.7. Research plan

The first part of the research focused on selection of materials for fabricating the devices. This was accomplished by preparing thin films of several polymers, alone or in combination, and studying their degradation rates. As can be seen from section 1.6 and Figure 1, the different parts of the implant require different degradation rates, i.e., the walls that constitute the chambers should have the slowest degradation rates, the lids should have degradation rates such that they effect pulsatile release of 5-FU in 0, 4, 8, and 12 days, and lastly, the diclofenac containing implant should erode at a rate so as to provide continuous release. The results from the film degradation studies provided a basis for selection of materials for prototype fabrication using the TheriForm™ process. For practical reasons, the prototypes containing 5-FU and diclofenac were fabricated separately. Whenever possible, each prototype contained a non-drug containing portion to mimic the presence of the part that would contain the second drug since the ultimate intention is to combine the two implants into a single multi-drug device once each part has been separately optimized. Analytical methods were developed to assay the drugs. These methods were then used to determine the *in vitro* rate of drug release from the different prototypes fabricated using TheriForm™ process. A flow chart of the overall research strategy is presented below:



Please refer to Appendix C for a Gantt chart of the performance schedule.

2. RESEARCH

2.1. Analytical method development

Diclofenac sodium (diclofenac, DC) is a non-steroidal anti-inflammatory drug. Several methods are known for the determination of diclofenac, including thin-layer chromatography (TLC), gas chromatography (GC), and high-performance liquid chromatography (HPLC).[10, 11] TLC methods usually lack the sensitivity required for the determination of diclofenac in clinical samples and GC methods, although sensitive and selective, require extensive sample work-up, including derivatization. HPLC methods for the quantification of diclofenac using UV, fluorescence, and electrochemical detection are well documented. Similarly, 5-fluorouracil (5-FU), a fluorinated anti-metabolite of the pyrimidine base uracil, also has several literature references for quantitative determination as it has been the subject of research and application as an anticancer and anti-proliferative drug during the last two decades.[12, 13] However, none of the available analytical methods describe an application for both 5-FU and diclofenac when formulated in a biodegradable polymer matrix. The first objective was to develop separate assays for determining the rate of drug release from 5-FU implants and diclofenac implants. Since the ultimate aim of the project is to combine both biodegradable implants in a single device capable of prescriptive release of both 5-FU and diclofenac, the second objective was to develop an assay for simultaneous quantitative determination of both drugs.

The following methods were developed and tested for linearity, precision, specificity, and sensitivity:

1. An isocratic high-performance liquid chromatography (HPLC) method for the quantitative determination of 5-FU in polymeric biodegradable implants.

2. An isocratic HPLC method for the quantitative determination of diclofenac in polymeric biodegradable implants.

3. An isocratic HPLC method for the quantitative determination of both 5- FU and diclofenac in polymeric biodegradable implants.

Instruments: The methods were developed in two binary HPLC systems (Hewlett-Packard (HP) 1100 series) equipped with an HP ChemStation data acquisition system (running software version A.04.02). Both systems were configured with a thermostatic control column compartment. Detection was accomplished with either an HP variable wavelength detector (VWD) or diode array detector (DAD). The assays were performed using either μBondapack C18, 10μ, 3.9x300 mm columns (Waters) or LiChrosorb RP-18, 5μ, 4.6x250 mm columns (Alltech). In each case, a compatible phase, pre-column cartridge was employed.

Reagents and solvents: Diclofenac sodium (99%) was obtained from Sigma (St. Louis, MO). 5-Fluorouracil, USP/NF grade (dried assay 98.5-102.0%) was obtained from Spectrum (New

Brunswick, NJ). All other reagents were of analytical or spectroscopic grade and were used as received. All HPLC solvents were analytical grade materials and were used as received. Water was HPLC grade produced in-house with a NANOpure system (Barnstead).

2.1.1. Diclofenac assay

2.1.1.1. Experimental

Standard and sample preparation: Standard stock solution was prepared by weighing 10 mg of diclofenac into a 100 ml volumetric flask and diluting to volume with methanol. Solutions for standard curve determination were prepared at concentrations of 0.5, 1.0, and 20.0 μg/ml by appropriate dilution of the standard stock solution.

Assay procedure: The system was equilibrated for 1h at the following conditions: Column, μBondapack C18, 10μ, 3.9 x 300 mm (Waters); Guard Column, μBondapack C18 (Alltech); Temperature, 25°C; Detection, UV at 280 nm (VWD); Buffer: 45 mM phosphate buffer, pH 7.0 (6.12 g KH_2PO_4 in 1000 ml H_2O, adjusted to pH 7.0 with 1.0 M KOH). 20 μl samples were manually injected and run at the above conditions with the changes in mobile phase as indicated in each section. Data was collected for 10 min and the peak area was analyzed using the standard integration method of the HP ChemStation system. Retention time for diclofenac was 5.2 min.

Linearity: Linearity test was performed for the four different mobile phase combinations:

Mobile Phase A: 60% Buffer, 40% (CH_3CN/THF, 7:3), 1.0 ml/min flow rate.
Mobile Phase B: 55% Buffer, 45% CH_3CN, 1.0 ml/min flow rate.
Mobile Phase C: 65% Buffer, 35% CH_3CN, 1.0 ml/min flow rate.
Mobile Phase D: 65% Buffer, 35% CH_3CN, 1.2 ml/min flow rate (same as mobile phase C but different flow rate)

Precision: System precision test for diclofenac was determined with 2.0 μg/ml and 20 μg/ml standard solutions.

Specificity: The following samples were prepared to check for method specificity:

A. Drug release medium (71 mM sodium phosphate buffer saline).
B. 118 mg of the polymer matrix (poly lactic-co-glycolic acid (PLGA) 50:50, 50KDa) in 10 ml incubated for 1 day at 37°C (sample name: pla1, 1 day)
C. 118 mg of the polymer matrix (PLGA 50:50, 50KDa) in 10 ml incubated for 4 days at 37°C (sample name: pla1, 4 days)
D. 118 mg of the polymer matrix (PLGA 50:50, 50KDa) in 10 ml incubated for 7 days at 37°C (sample name: pla1, 7 days)

E. A 150 mg placebo device (PLGA 50:50, 50KDa) in 100 ml of mobile phase, to simulate the sample preparation procedure (sample name: pla2)
F. Mobile phase, 45 mM phosphate buffer:CH_3CN (7:3)

Sensitivity: The lowest concentration of diclofenac detectable by the assay method was determined.

2.1.1.2. Results and discussion.

Linearity: System linearity was excellent in all cases for a concentration range of 0.5 μg/ml to 20.0 μg/ml. Mobile phase conditions C and D were selected for prototype testing because of their linearity (see Figure 2) and short diclofenac retention time. Mobile phase system A (diclofenac retention time 5.12 min) was discarded because THF was not compatible with some of the HPLC system components. Condition B (diclofenac retention time 3.5 -4.0 min) was not used because an interference by the polymer-placebo was observed between 3.0 -3.5 min. Also, the diclofenac peak showed broadening and tailing in this mobile phase. The plots of peak area vs. diclofenac concentration (μg/ml) for mobile phase conditions C and D showed linear behavior in the 4.0-18.0 μg/ml range, with linear regression coefficients (R^2) of 1 and 0.9999, respectively.

Precision: System precision test for diclofenac was determined with 2.0 μg/ml and 20 μg/ml standard solutions. Relative standard deviation values (RSD) were 0.33% and 0.60% respectively (see table and graph in Figure 3).

Specificity: Interference in all tests was negligible, with % area values of less than 1% in each case (see Table 1).

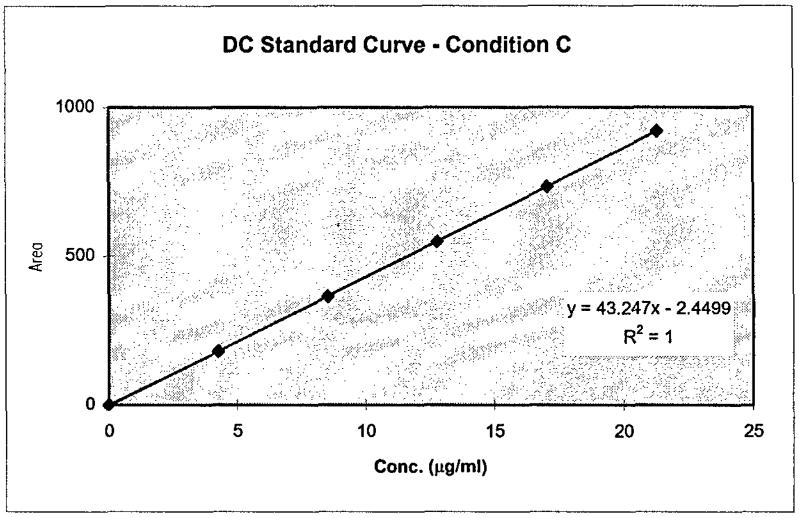
Sensitivity: For the purpose of these studies the lowest concentration tested was 0.5 μg/ml. No studies were performed for impurity and decomposition products assay.

2.1.1.3. Conclusions

An assay method for quantitative analysis of diclofenac was developed. The data indicates that the method is linear, precise, specific, and sensitive. The method was successfully used for determining drug content uniformity and drug release from implant prototypes containing diclofenac.

(A) Mobile Phase Condition C

Conc. (ug/ml)	Area Units
0.00	0.00
4.26	181.15
8.52	363.76
12.78	548.56
17.04	734.95
21.30	920.40



(B) Mobile Phase Condition D

Conc. (ug/ml)	Area Units
0.00	0.00
4.26	146.64
8.52	299.80
12.78	450.92
17.04	594.40

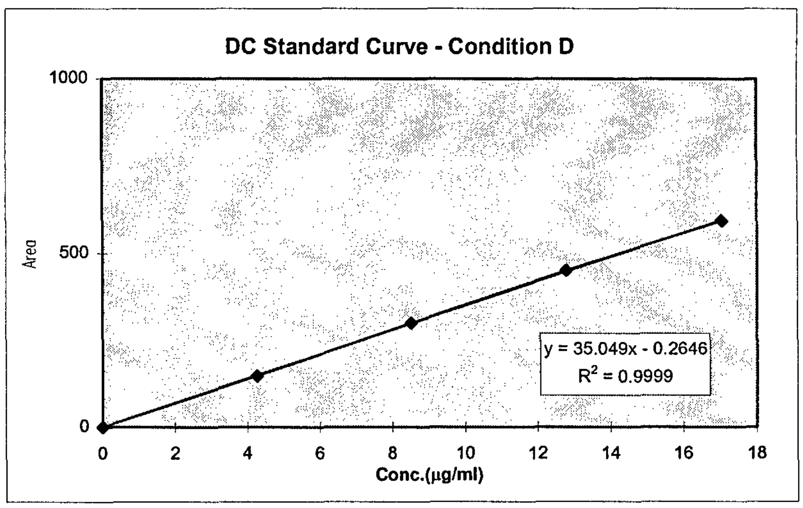
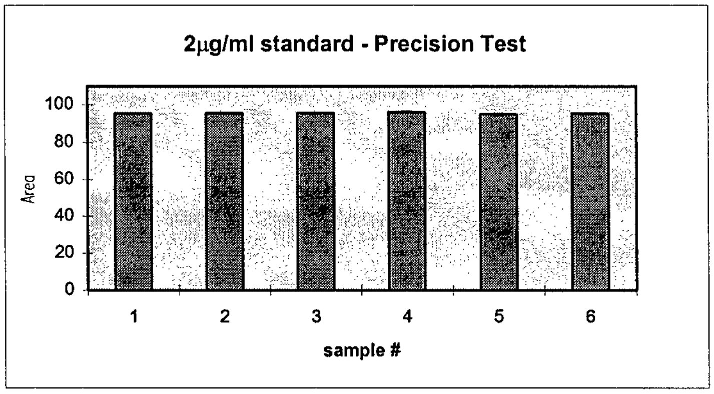


Figure 2. Diclofenac standard curves for (A) mobile phase condition C and (B) for mobile phase condition D.

(A) Precision test with 2.0 μg/ml diclofenac solution

Sample *2 ug/ml*	Area Units
1	95.45
2	95.67
3	95.71
4	96.15
5	95.11
6	95.50
RSD	0.33%



(B) Precision test with 20.0 μg/ml diclofenac solution

Sample *20 ug/ml*	Area Units
1	942.86
2	933.40
3	930.50
4	933.85
5	923.94
6	931.51
RSD	0.60%

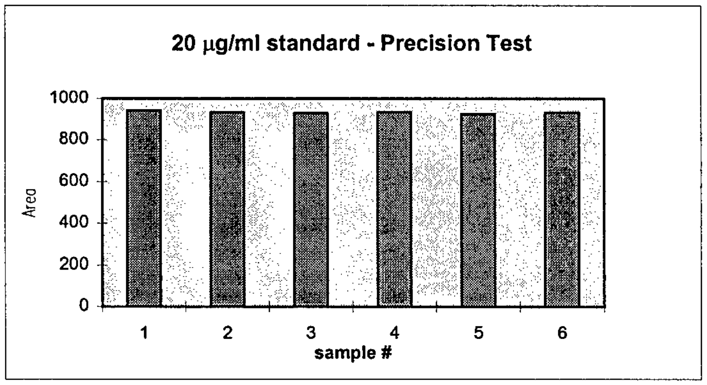


Figure 3. **Precision test for diclofenac assay using (A) 2.0 μg/ml diclofenac solution and (B) 20.0 μg/ml diclofenac solution**

Table 1. Specificity test for diclofenac assay

Sample Name	Area Units	Interference (%)	Ret. Time (min)
Pure PBS	0.00	0.00	N/A
pla1,1days	2.12	0.61	5.40
pla1,4days	1.85	0.53	5.40
pla1,7days	1.72	0.49	5.40
pla 2	0.59	0.17	5.40
Mobile Phase	0.00	0.00	N/A

Percent interference based on 10 μg/ml solution (area = 350.28 units).
Where pla1 is PLGA (50:50) 50KDa polymer material and
pla2 is placebo device made with PLGA (50:50), 50KDa.

2.1.2. 5-Fluorouracil assay

2.1.2.1. Experimental

Standard and sample preparation: 5-FU standard stock solution was prepared by weighing 20 mg of 5-FU into a 100 ml volumetric flask and diluting to volume with H_2O. Solutions for standard curve determination were prepared at concentrations of 1.0, 2.0, 4.0, 5.0, 6.0, 8.0, 10.0, 20.0, 30.0, 40.0, 60.0, 80.0, and 100 μg/ml by appropriate dilution of the stock standard solution.

Assay procedure: The system was equilibrated for 1h at the following conditions: Column, LiChrosorb RP-18, 5μ, 4.5x250 mm (Alltech); Guard Column, LiChrosorb RP-18 (Alltech); Temperature, 30°C; Detection, UV at 254 nm (DAD); Mobile Phase, Neat HPLC-grade water from the in-house systems filtered through a 0.22 μm nylon membrane (Magna-R, MSI, Westboro, MA); Run time, 8 min. Samples from the 5-FU prototypes release study were loaded into 1 ml crimped vials. Samples were injected with the autosampler (HP 1100 series). The injection volume was set to 20 μl. A needle wash step was included in the method to prevent cross-sample contamination. Finally, the samples were run at the above conditions with the changes in mobile phase as indicated in each section. Data was collected for 10 min and the peak area was analyzed using the standard integration method of the HP ChemStation system.

Linearity: A linearity test for 5-FU was performed at three different conditions of flow rates and/or concentration ranges to cover the anticipated, wide concentration range of drug release samples of the 5-FU pulsatile release-prototypes.

Precision: A system precision test for 5-FU was determined with 1.0, 10.0, 40.0, 60.0, and 80.0 μg/ml standard solutions.

Sensitivity: The lowest concentration of 5-FU detectable by the assay method was determined.

2.1.2.2. Results and discussion

Linearity: System linearity was good in all systems over a concentration range of 1.0 to 80.0 μg/ml. The plots of peak area vs. diclofenac concentration (μg/ml) showed linear behavior in the 1.0 - 10.0 μg/ml, 1.0 - 30.0 μg/ml, and 40.0 - 80.0 μg/ml ranges, with linear regression coefficients (R^2) of 0.9994, 0.9998 and 0.9993, respectively (see Figure 4).

Precision: System precision test for 5-FU was conducted using 1.0, 10.0, 40.0, 60.0, and 80.0 μg/ml standard solutions. Relative standard deviation values (RSD) were 0.45%, 0.14%, 0.08%, 0.03% and 0.05%, respectively (see Figure 5).

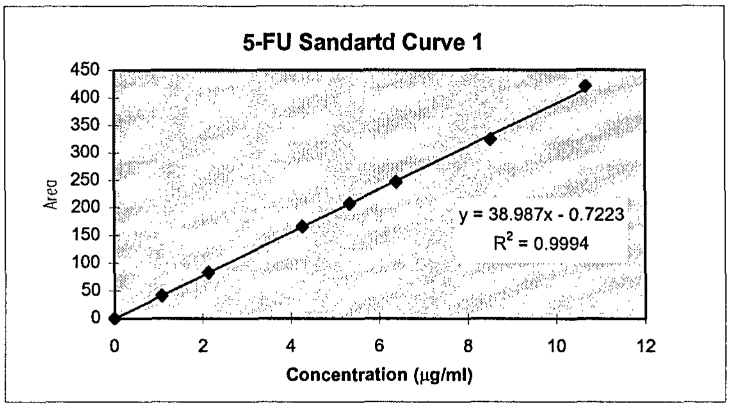
Sensitivity: For the purpose of these studies the lowest concentration tested was 1.0 μg/ml. No studies were performed for impurity and decomposition products assay.

2.1.2.3. Conclusions

An assay method for quantitative analysis of 5-FU was developed. The data indicates that the method is linear, precise, specific, and sensitive. The method was successfully used for determining drug content uniformity and drug release from implant prototypes containing 5-FU.

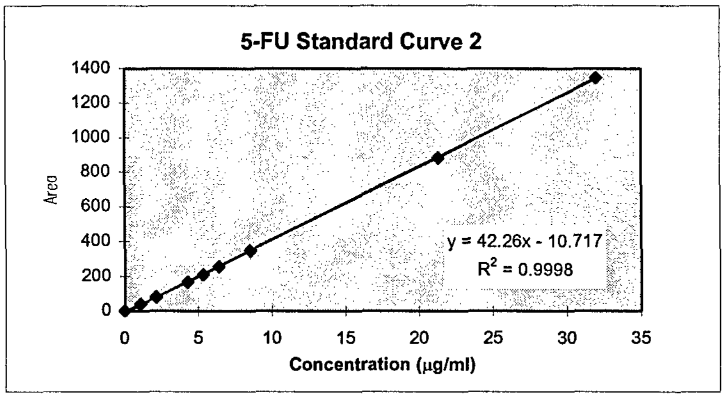
(A) 5-FU standard curve at flow rate: 1.2 ml/min

Conc.(ug/ml)	Area Units
0.00	0.00
2.13	82.88
4.26	166.16
5.33	207.55
6.39	246.97
8.52	324.59
10.66	420.56



(B) 5-FU standard curve at flow rate 1.0 ml/min

Conc.(ug/ml)	Area Units
0.00	0.00
1.07	39.49
2.13	79.65
4.26	164.25
5.33	208.63
6.39	255.56
8.52	347.30
21.31	883.69
31.97	1347.21



(C) 5-FU standard curve at flow rate 1.0 ml/min for high sample load (40.0 - 80.0 μg/ml).

Conc. (ug/ml)	Area Units
0.00	0.00
40.00	1593.00
60.00	2373.00
80.00	3078.00

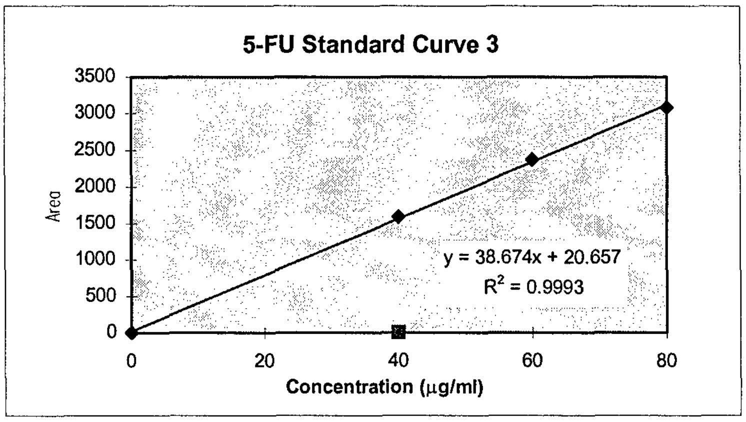
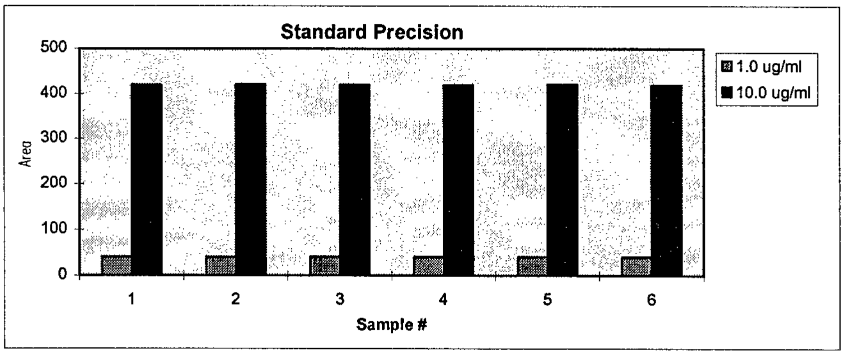


Figure 4. **5-FU standard curves for (A) 1.2 ml/min, (B) 1.0 ml/min and (C) 1.0 ml/min high sample load (40.0 - 80.0 μg/ml).**

1µg/ml sample	Area Units	10µg/ml sample	Area Units	40µg/ml sample	Area Units	60µg/ml sample	Area Units	80µg/ml sample	Area Units
1	40.85	1	420.43	1	1591.10	1	2371.60	1	3075.90
2	40.82	2	421.29	2	1592.90	2	2373.00	2	3076.40
3	41.17	3	420.45	3	1592.80	3	2373.20	3	3077.80
4	41.35	4	419.89	4	1594.50	4	2372.90	4	3079.30
5	41.11	5	421.37	5	1594.50	5	2373.10	5	3079.60
6	41.14	6	419.91	6	1592.40	6	2374.10	6	3079.20
RSD	0.45%	RSD	0.14%	RSD	0.08%	RSD	0.03%	RSD	0.05%



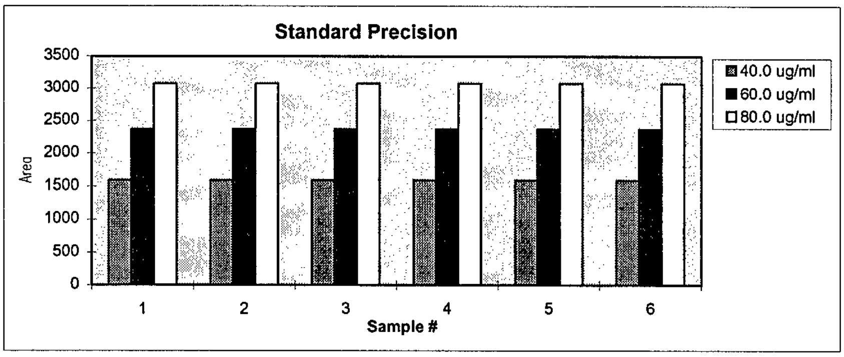


Figure 5. **5-FU standard precision test for low (1.0 and 10.0 µg/ml) and high (40.0, 60.0, 80.0 µg/ml) sample loads.**

2.1.3. Combined 5-FU and diclofenac assay

2.1.3.1. Experimental

Standard and sample preparation: 5-Florouracil (5-FU) standard stock solution was prepared by weighing 20 mg of 5-FU into a 100 ml volumetric flask and diluting to volume with H_2O. Diclofenac sodium (diclofenac, DC) standard stock solution was prepared by weighing 10 mg of diclofenac into a 100 ml volumetric flask and diluting to volume with methanol. Solutions for standard curve determination were prepared by 1:10 mixing of the 5-FU and diclofenac standard stock solutions, respectively, followed by appropriate dilutions (the final drug concentrations of the solutions for standard curve determination are listed in Table 2).

Assay procedure: The system was equilibrated for 1h at the following conditions: Column 1, µBondapack C18, 10µ, 3.9 x 300 mm (Waters) with guard column, µBondapack C18 (Alltech); or, Column 2, LiChrosorb RP-18, 5µ, 4.5x250 mm (Alltech) with guard column, LiChrosorb RP-18 (Alltech); Temperature, 30°C; Detection, UV at 280 nm (DAD); Mobile Phase, 70 % 40 mM sodium phosphate buffer and 30% CH_3CN, filtered through a 0.22 µm nylon membrane (Magna-R, MSI, Westboro, MA); Run time, 8 min.

Samples from the release studies were loaded into 1 ml crimped vials. Samples were injected with the autosampler (HP 1100 series). The injection volume was set to 20 µl. A needle wash step was included in the method to prevent cross-sample contamination. Finally the samples were run at the above conditions.

Linearity: A linearity test was performed for the three different settings with varying flow rates and/or concentration ranges.

Precision: The method precision was tested for drug concentrations shown in the Table 2.

Specificity: The placebo interference for the method was tested with a sample from 5-FU Prototype 3.

2.1.3.2. Results and discussion

Assay: Samples were run at the above conditions. Data was collected for 8-10 min and the peak area was analyzed using the standard integration method of the HP ChemStation system. Retention times were 1.9 - 2.0 min for 5-FU, and 7.0 - 7.5 min for diclofenac.

Linearity: System linearity is good for both drugs systems over a concentration range of 1.0 to 60.0 µg/ml. The plots of peak area vs. drug concentration (µg/ml) for both 5-FU and diclofenac show linear behavior in the 1.0 - 60.0 µg/ml range, with linear regression coefficients (R^2) of 1 and 0.9998, respectively (see Figure 6).

Table 2. Precision test for 5-FU and diclofenac in the combined HPLC assay.

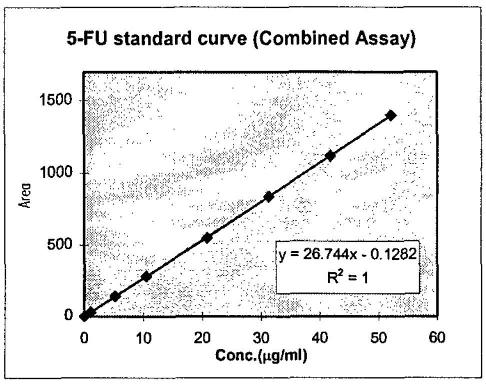
Diclofenac				5-FU			
Std conc. (ug/ml)	**Area Units**			**Std conc. (ug/ml)**	**Area Units**		
10.128	285.06	**Average**	284.608	2.118	54.37	**Average**	54.470
	285.03	**STDev**	0.329		54.50	**STDev**	0.067
	284.33	**RSD**	0.12%		54.55	**RSD**	0.12%
	284.34				54.37		
	284.52				54.44		
	284.82				54.50		
1.013	28.86	**Average**	28.703	0.212	5.57	**Average**	5.494
	28.77	**STDev**	0.200		5.35	**STDev**	0.100
	28.48	**RSD**	0.70%		5.56	**RSD**	1.82%
2.026	57.94	**Average**	57.948	0.424	11.09	**Average**	11.088
	58.15	**STDev**	0.161		11.07	**STDev**	0.012
	57.76	**RSD**	0.28%		11.10	**RSD**	0.11%
4.051	115.75	**Aveaage**	115.585	0.847	22.14	**Average**	22.087
	115.64	**Average**	0.157		22.05	**STDev**	0.036
	115.37	**RSD**	0.14%		22.07	**RSD**	0.16%
6.077	174.06	**Average**	174.638	1.271	33.20	**Average**	33.578
	175.02	**STDev**	0.414		33.75	**STDev**	0.269
	174.83	**RSD**	0.24%		33.79	**RSD**	0.80%
8.102	234.76	**Average**	235.051	1.694	45.12	**Average**	45.120
	234.98	**STDev**	0.271		45.07	**STDev**	0.040
	235.41	**RSD**	0.12%		45.17	**RSD**	0.09%
20.256	582.46	**Average**	582.981	4.236	112.48	**Average**	112.510
	583.04	**STDev**	0.403		112.48	**STDev**	0.041
	583.45	**RSD**	0.07%		112.57	**RSD**	0.04%
30.384	879.34	**Average**	879.298	6.354	169.26	**Average**	169.389
	879.18	**STDev**	0.084		169.37	**STDev**	0.116
	879.37	**RSD**	0.01%		169.54	**RSD**	0.07%
40.512	1176.99	**Average**	1178.230	8.472	226.51	**Average**	226.718
	1178.09	**STDev**	1.074		226.50	**STDev**	0.301
	1179.61	**RSD**	0.09%		227.14	**RSD**	0.13%
50.640	1454.66	**Average**	1455.523	10.590	280.19	**Average**	280.496
	1455.22	**STDev**	0.856		280.56	**STDev**	0.227
	1456.69	**RSD**	0.06%		280.74	**RSD**	0.08%

5-Fu Reproducibility		
Conc.	Area	Average
ug/ml	Units	area
1.04	27.79	27.84
	27.86	STDev
	27.85	0.04

Linearity	
Conc.	Area
ug/ml	Units
0.00	0.00
1.04	27.84
5.22	140.63
10.44	282.43
20.88	550.97
31.32	838.67
41.76	1116.32
52.20	1397.65

Diclofenac Reproducibility		
Conc.	Area	Average
ug/ml	Units	area
1.11	33.06	33.04
	33.10	STDev
	32.97	0.07

Linearity	
Conc.	Area
ug/ml	Units
0.00	0.00
1.11	33.04
5.55	161.80
11.11	326.64
22.21	649.72
33.33	976.85
44.42	1296.61
55.53	1652.20



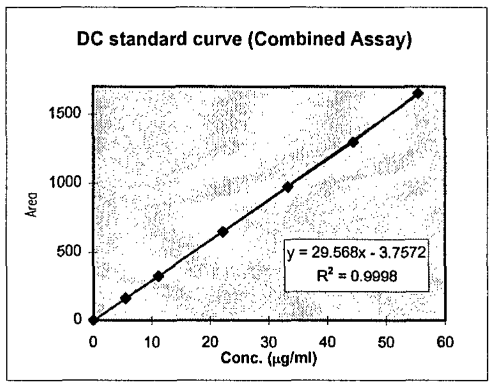


Figure 6. Linearity and reproducibility tests for 5-FU and diclofenac in the combined HPLC assay.

Precision: The precision for the method was tested for the concentrations shown in the Table 2. The results indicate that the combined assay method is both precise and reproducible in the concentration range tested.

Specificity: The interference from a placebo sample (5-FU Prototype 3) in all tests was negligible, with % area values of less than 1% in each case. No placebos of the diclofenac prototype were tested at this time since the polymer matrix was the same as the 5-FU prototype.

2.1.3.3. Conclusion

An HPLC method for the combined quantitative analysis of 5-FU and diclofenac was developed. The data indicates that the method is linear, precise, specific, and sensitive. The method was successfully used for determining drug content uniformity and drug release from implant prototypes containing 5-FU and diclofenac.

Note: The raw data for the analytical method development is described in the following Therics' laboratory notebooks:

1. Notebook 010, pages 012, 016-018, 021-024, 026-029, 032-034, 040-050, 063, 066-100.

2. Notebook 017, pages 001-029, 031-033, 036-041, 044-050, 054-056, 058-060.

2.2. 5-Fluorouracil implant fabrication and testing

2.2.1. Experimental

2.2.1.1. Film studies

Several polymeric materials were selected for film degradation studies. Different copolymers of the poly-lactides and poly-glycolides of a wide range of molecular weights were studied. A number of polyanhydrides were also tested as candidates for the erosion layers. Thin films of the polymers were prepared by the technique of solvent casting using chloroform as the solvent. This was a quick and easy method for initial screening of the degradation rates of various polymer combinations. Two sets of films were cast using the same polymer material but with thicknesses of 300 μm and 600 μm, respectively. The concentration of the polymeric solution was calculated based on the theoretical density of the solute polymers to yield the desired thicknesses. The resulting films were dried at room temperature for eight days, followed by overnight drying in vacuum oven. The films were cut into approximately 1cm x 1cm pieces and then placed in 10 ml of 10 mM phosphate buffered saline (PBS) solution. The vials were then subjected to continuous shaking in an incubator maintained at 37°C. The samples were monitored for change in integrity and appearance.

2.2.1.2. Prototype devices

Powder Milling: A powder milling process was necessary to prepare the as-received polymer powders for the TheriForm™ process. A cooling circulation bath was used to keep the analytical mill (Janke & Kunkel GmbH & Co., 20,000 rpm) at -15°C during the milling to keep the operation temperature well under the glass transition temperature of the polymers. Resulting powders were then classified using sieves (W.S.Tyler, USA Standard Testing Sieve, 100 mesh and 200 mesh). Particles in the range of 75μm to 150μm were used to build all of the 5-FU prototypes.

Default fabrication material and parameters: Poly (lactic-glycolic acid) (PLGA) (50:50) 50KDa (Boehringer Ingelheim, RG504, Lot 34015), was selected as the base material for the fabricating the framework of the prototypes based on the favorable results from the film degradation studies. Unless stated otherwise, poly (fatty acid dimer: sebacic acid) (P(FAD:SA)) (50:50) 51KDa (Hebrew University of Jerusalem, Lot TD3-10b), was used to build lids onto the PLGA boxes. All polymers were milled and sieved to obtain powders in the size range of 75μm to 150μm. Chloroform (Fisher Scientific, HPLC Grade, Lot 970035) was used as the binder to fabricate all of the prototype devices. Flow rate of the chloroform jet was kept at 1.2 cc/min throughout the build processes. Fast axis scan speed was fixed at 1.5m/sec.

A schematic of the prototype design is shown in Figure 1. The base of the devices was fabricated by building five 170μm layers, for a total thickness of 850μm. Twelve 170μm layers of walls were built on top of the base for the total thickness of 2.04mm. The default process parameters used for the fabrication of the prototype devices are listed in Table 3. Deviations from these conditions will be explained individually in the following section and the rationale for the changes will be discussed in detail.

Drug loading: Drug loading of each prototype devices was accomplished by depositing 16mg/ml solution of 5-FU in 0.1N NaOH solution. Each reservoir was manually loaded with 10μl of the solution resulting in the total of 160 μg of 5-FU per compartment. Loaded devices were subjected to drying in a closed fume hood for 24 hours before constructing the polymer lids.

A number of different prototype devices were fabricated for the release of 5-FU. The following sub-sections describe the uniqueness of designs, fabrication strategies, and build parameters for each of the prototypes.

5-FU Prototype 1 (5FUP1): PLGA (50:50) 50KDa was selected as the base material for the fabrication of 5-FU Prototype 1 (5FUP1). The fabrication parameters were the same as the default parameters described above. Walls of 5FUP1 were built by printing 4 lines of chloroform jet, each separated by 170μm onto PLGA powder. Devices were loaded with 5FU according to the above described procedure. No lids were built on top of these devices. These devices were designed to simulate the condition in which all of the lid layers have eroded away. Poor solubility of 5-FU, poor wetting characteristics of the PLGA, and the stagnant dissolution medium around the devices were the main reasons to study release rates from an open device.

5-FU Prototype 2 (5FUP2): Materials and other fabrication parameters used for 5FUP2 are identical to that described above. Unlike 5FUP1 devices, 5FUP2 had four compartments separated by two orthogonal inner-walls. These walls were fabricated by making a simple modification to the process controlling program written for 5FUP1. Figure 7 shows a batch of 5FUP2 devices as they were completed on the powder bed.

5-FU Prototype 3 (5FUP3): The materials and fabrication conditions used for 5FUP3 are identical to that of 5FUP1 with the exception that the polymer lids were fabricated on top of the open boxesto provide closure. P(FAD:SA) powders and chloroform as the binder were used to build these lids. Default build parameters as shown in Table 3 were used to fabricate 5FUP3 devices with an exception of the lid portion. A double-printing technique was used to fabricate the P(FAD:SA) lid layers. Three different sets of 5FUP3 devices with 3, 5, or 7 P(FAD:SA) lid layers were fabricated and tested.

5-FU Prototype 4 (5FUP4): The amount of chloroform printed onto the PLGA and P(FAD:SA) powders was doubled in 5FUP4 to ensure the formation of defect-free devices walls. This increase in binder was achieved by double-printing the chloroform jet onto the powder. After the walls were fabricated with PLGA, the devices were loaded with 5FU. The top surface of the walls where the P(FAD:SA) lid makes the contact was pre-saturated with chloroform to enhance bonding between the two polymer systems. P(FAD:SA) powder was then used to build the lid layers. Line spacing of 85μm was used to build the top lid portion of the device. Devices with 2, 4, and 6 layers of the P(FAD:SA) layers were constructed and tested.

Table 3. Default process parameters used to fabricate prototype 5-FU devices.

Process Parameter*	Values
Flow Rate	1.2 ml/min
Print Speed	1.5 m/sec
Line Spacing	170 μm
Layer Height	170 μm
Number of Passes	1

* See Appendix A and Appendix B for a detailed description of the process and glossary of terms, respectively.

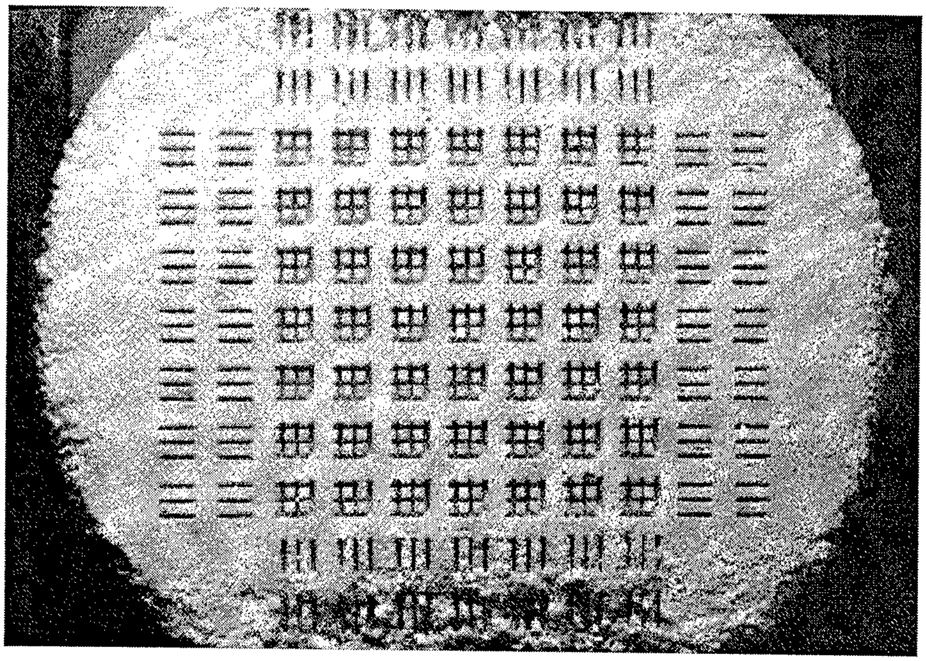


Figure 7. Rotational powder bed with completed 5-FU Prototype 2 (5FUP2) devices.

5-FU Prototype 5 (5FUP5): 5FUP5 devices were built with thicker walls than the previous prototypes. Eight lines, separated from each other by 170μm, were printed to form each wall in these devices. The double-printing technique described above was also used for 5FU5 devices. The top surfaces of the devices were again pre-saturated with chloroform in preparation for lid construction and the P(FAD:SA) lids were built with 85μm line spacing. Prototype devices with 2, 3, and 4 layers of P(FAD:SA) were fabricated and tested.

5-FU Prototype 6 (5FUP6): 5FUP6 devices were fabricated entirely with PLGA. These devices were constructed in an identical condition as the 5FUP5 with an exception of the lid material and the line spacing used to print the lid. Line spacing used for 5FUP6 lid was 170 μm. Prototype devices with 2, 3, and 4 layers of PLGA lids were fabricated and tested.

2.2.1.3. Drug release study

Prototype 5-FU devices were placed in 20 ml glass scintillation vials with 10 ml of phosphate buffered saline (PBS) solution at pH 7.4. Polyethylene caps were used to seal the scintillation vials. Temperature of the incubator was kept at 37°C and the dissolution medium was kept undisturbed to simulate the intravitreal condition. The PBS solution was completely drawn and collected at predetermined time intervals for assay and sample vials were replenished with 10 ml of fresh PBS. Release samples were analyzed by HPLC methods described in sections 2.1.2 and 2.1.3.

2.2.2. Results and discussion

2.2.2.1. Film studies

The film degradation study results are summarized in Table 4. Most of the PLGA combinations yielded durable films that did not exhibit considerable degradation within the initial 30 days of the study. This type of durability was required to form the framework of the 5-FU containing portion. The device design illustrated in Figure 1 mandates that the bottom and side walls of the device be slow degrading, acting as an impermeable barrier for 5-FU. PLGA (50:50, 50 KDa) was selected to fulfill this function in the prototype devices based on favorable results from the film degradation studies. Top lid materials for the 5-FU chambers, however, need to erode away fast enough to cause a designed sealing failure and subsequent burst of 5-FU. Polyanhydrides are known to exhibit surface degradation behavior which is ideal for lids. P(FAD:SA) (50:50, 51 KDa) films of different thickness, 300 μm and 600 μm, respectively, degraded between 5 to 7 days which is ideal for the application.

2.2.2.2. Prototype devices and release studies

5-FU Prototype 1 (5FUP1): 5FUP1 devices were designed to test the release of 5FU from the chambers in the absence of lid layers. Results from the release study are illustrated in Figure 8. As evident from Figure 8, 5-FU released immediately from these prototypes within 3 hours. This result indicated that the dissolution of 5-FU in the surrounding medium would not be the rate limiting step for these devices at this concentration. Diffusion through the loose PLGA powder inside of these devices also does not seem to pose any problem for the release of 5-FU. This result signifies the fact that one may achieve the pulsatile release profile once an appropriate erosion layer is placed on the top of these devices.

5-FU Prototype 2 (5FUP2): A completed 5FUP2 device is shown in Figure 9. Microstructure analysis by SEM revealed several important characteristics about the devices. Figure 10 is taken from the top center of a 5FUP2 device. The cross in the center represents the internal walls of the device that separates the four chambers. The PLGA powders in the chambers appear to be loose and unbound while the walls seem to be dense. Micrographs at higher magnification (100X) reveal some more detail about each region as shown in Figure 11 and Figure 12.

Imperfections present in the walls may allow an undesired channeling of 5FU through the side walls of the device. However, the small pores that are shown in Figure 11 do not seem to be interconnected. This suggests that there should be no significant leakage. This issue is addressed in greater detail in later sections.

Table 4. Summary of the film degradation study results.

Material	Final Result
Film Study 1	
PLGA (75:25) 15 KDa 300 μm	Curled into an amorphous blob after one day
PLGA (75:25) 60 KDa 300 μm	Was still a sheet after twenty nine days
PLGA (50:50) 50 KDa 300 μm	Was still a sheet after twenty nine days
P(DL)LA 5-10 KDa:PLGA (75:25) 15KDa (1:1) 300 μm	Curled into an amorphous blob after one day
PLLA 2KDa:PLGA (75:25)15 KDa (1:1) 300um	Curled into an amorphous blob after one day
P(DL)LA 5-10 KDa: PLGA (75:25) 60 KDa (1:1) 300 μm	Was still a sheet after twenty nine days
PLLA 2KDa:PLGA (75:25)60 KDa (1:1) 300 μm	Grew fat and irregular in shape at twenty eight days
P(DL)LA 5-10 KDa: PLGA (50:50) 50 KDa (1:1) 300 μm	Broke apart and mostly dissolved after one day
PLLA 2KDa:PLGA (50:50) 50 KDa (1:1) 300 μm	Became a shrunken blob at twenty four days
PLGA (75:25) 15 KDa 600 μm	Curled into an amorphous blob after one day
PLGA (75:25) 60 KDa 600 μm	Was still a sheet after twenty nine days
PLGA (50:50) 50 KDa 600 μm	Was still a sheet after twenty nine days
P(DL)LA 5-10 KDa:PLGA (75:25) 15KDa (1:1) 600 μm	Too soft to be cut for this study
PLLA 2KDa:PLGA (75:25)15 KDa (1:1) 600 μm	Too soft to be cut for this study
P(DL)LA 5-10 KDa: PLGA (75:25) 60 KDa (1:1) 600 μm	Became a shrunken blob at twenty four days
PLLA 2KDa:PLGA (75:25)60 KDa (1:1) 600 μm	Became a shrunken blob at twenty four days
P(DL)LA 5-10 KDa: PLGA (50:50) 50 KDa (1:1) 600 μm	Broke apart and mostly dissolved after one day
PLLA 2KDa:PLGA (50:50) 50 KDa (1:1) 600 μm	Became a shrunken blob at twenty four days
Film Study 2	
PLGA (50:50) 50 KDa 300 μm	Curled into a tube at fifty seven days
PLGA (50:50) 4.5 KDa 300 μm	Curled up into a blob almost immediately
PLGA (75:25) 60 KDa 300 μm	A small blob at fifty seven days
PLGA (50:50) 50 KDa:PEG 8.6 KDa (1:1) 300 μm	No film formed, brittle deposits only
PLGA (50:50) 4.5 KDa:PEG 8.6 KDa (1:1) 300 μm	No film formed, brittle deposits only
Polyanhydride P(CPP)SA 300 μm	No film formed, brittle deposits only
PLGA (50:50) 50 KDa P(CPP)SA 300 μm	Still intact after fifty-seven days
PLGA (50:50) 50 KDa 600 μm	Curled at fifty-seven days
PLGA (50:50) 4.5 KDa 600 μm	Too soft to be cut for this study
PLGA (75:25) 60 KDa 600 μm	A small, curled blob at fifty seven days
PLGA (50:50) 50 KDa:PEG 8.6 KDa (1:1) 600 μm	No film formed, brittle deposits only
PLGA (50:50) 4.5 KDa:PEG 8.6 KDa (1:1) 600 μm	No film formed, brittle deposits only
Polyanhydride P(CPP)SA 600 μm	No film formed, brittle deposits only
PLGA (50:50) 50 KDa P(CPP)SA 600 μm	Still intact after fifty-seven days
Film Study 3	
PFAD:SA (50:50) 300 μm	Degraded between four to six days
PFAD:SA (50:50) 600 μm	Degraded after seven days
PRAM:SA (50:50) 300 μm	Degraded after seven days
PRAM:SA (50:50) 600 μm	Degraded between four to six days
PSA 300 μm	No film formed, only brittle deposits
PSA 600 μm	No film formed, only brittle deposits

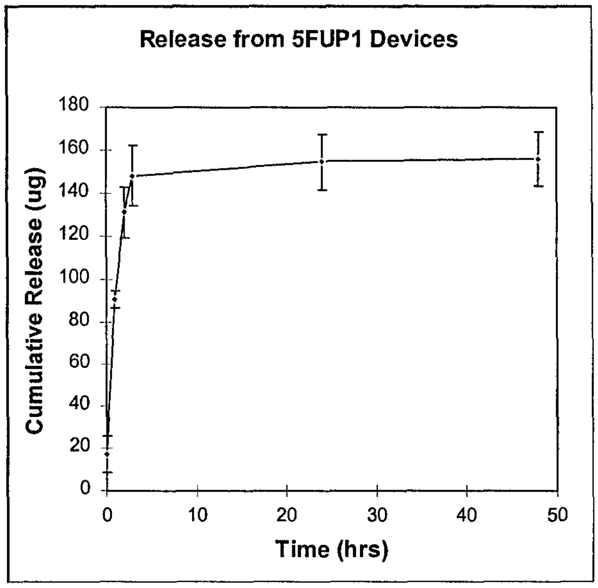


Figure 8. **Cumulative release from 5-FU Prototype 1 devices (n=4).**

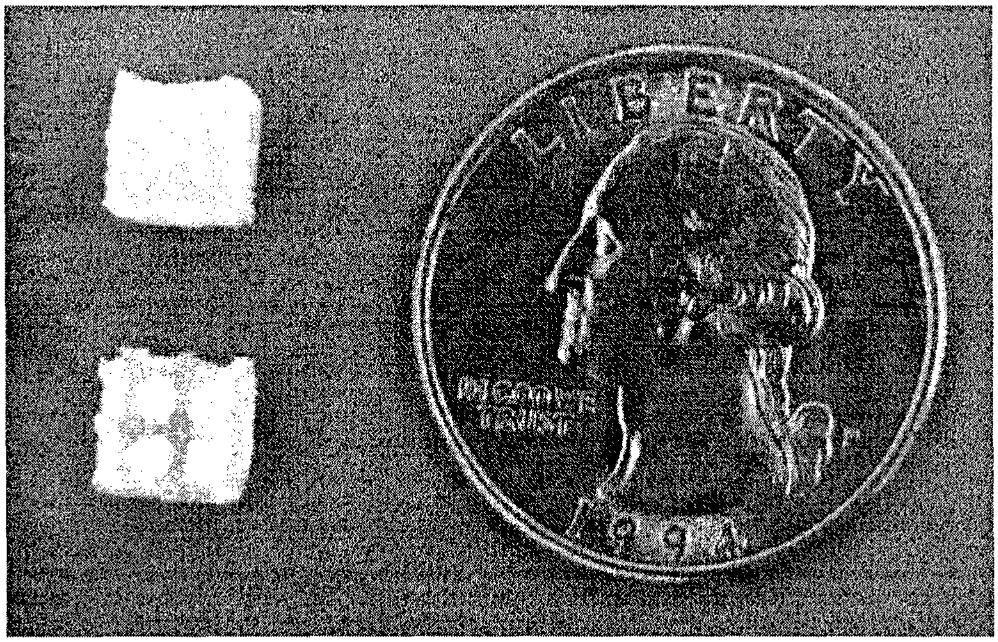


Figure 9. Prototype 5-FU devices fabricated by TheriForm™ process. 5FUP2 with four chambers is shown on the lower left. 5FUP4 with a polyanhydride lid is shown on upper left. The diameter of the quarter is approximately the same as that of a typical human eyeball.

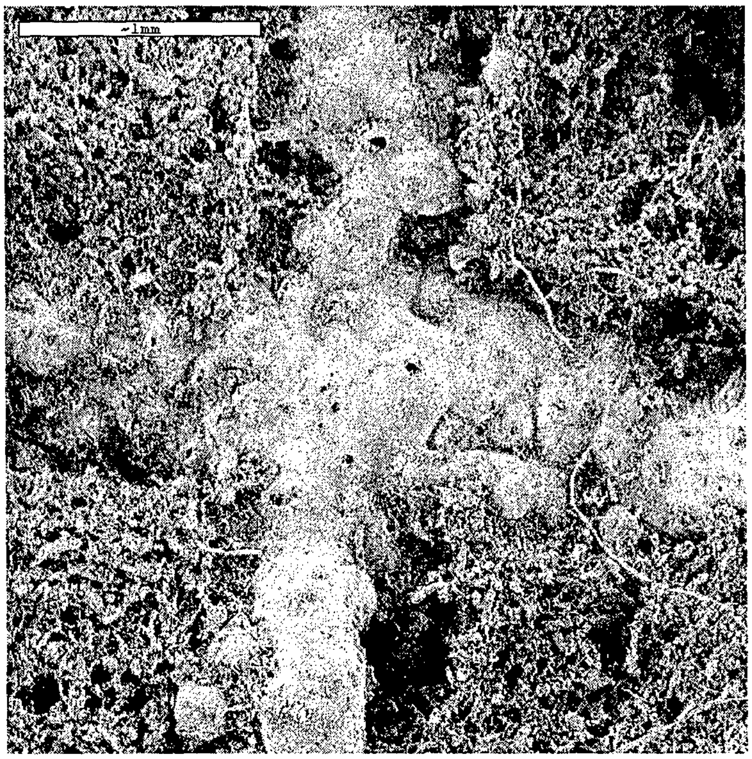


Figure 10. **Scanning electron micrograph (30X) taken at the top surface of a 5FUP2 device. Orthogonal walls separate the four chambers of the device.**

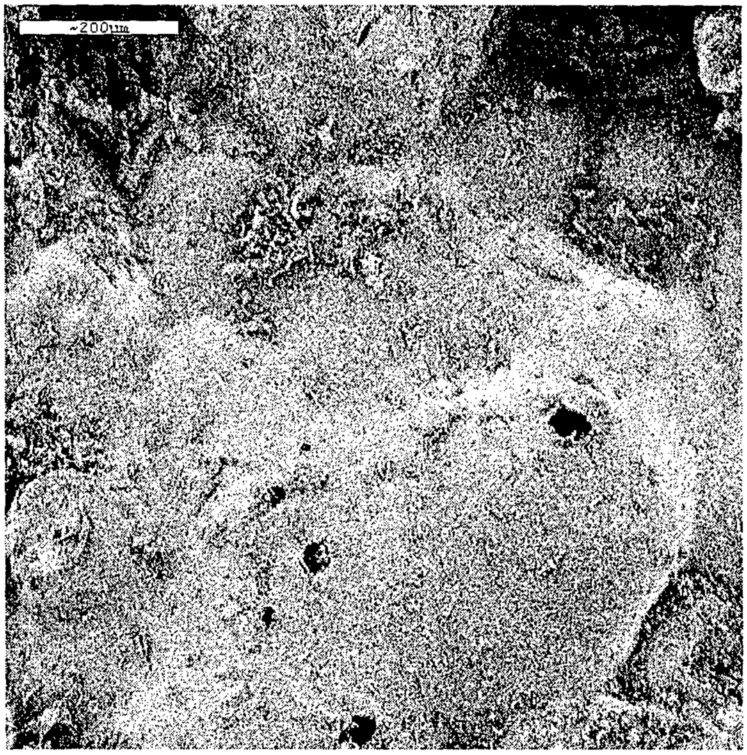


Figure 11. **SEM (100X) taken from the top surface of a 5FUP2 device. Close-up of the wall intersection. Few defects are present on the PLGA wall which appears very dense.**

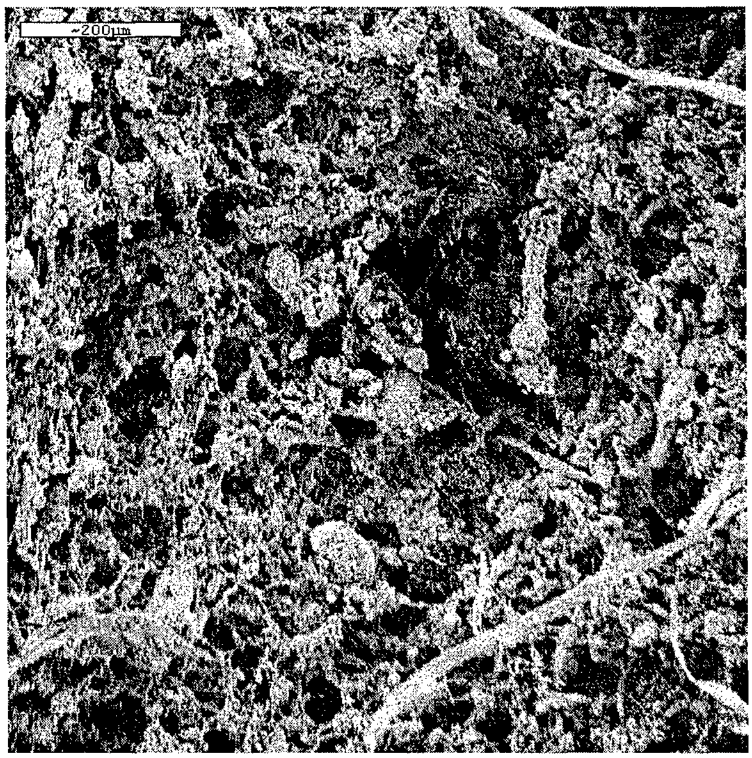


Figure 12. **SEM (100X) taken from the top surface of a 5FUP2 device. Close-up of the chamber shows the unbound PLGA powder.**

5-FU Prototype 3 (5FUP3): 5FUP3 devices were the first set of prototypes to incorporate lid layers to control the release characteristics of 5-FU. Three sets of devices, differing in the number of P(FAD:SA) lid layers, were fabricated and tested as described earlier. Figure 13 shows the release of 5-FU from these prototypes.

As evident from Figure 13, complete release of 5-FU from these devices occurred within the first few hours of the study. Release rate of 5-FU was identical in all three sets of devices with different lid layers. One possible cause for this type of release pattern is the presence of large defects which serve as escape routes for 5-FU molecules from the device chambers. Microstructure analysis confirmed the presence of defects at the PLGA and P(FAD:SA) interface as illustrated in Figure 14 and Figure 15. Also shown in Figure 16 is the top surface of the P(FAD:SA) layer and it is evident that the P(FAD:SA) particles dissolve partially and fuse to form the lid layer. It is also possible that this partial bonding of particles results in the interconnected pore channels through which 5-FU may release. Modifications in the fabrication process were required to eliminate these obvious defects in the lid layers as well as the delamination at the box-lid interfaces. Strategies and modifications for the next set of prototypes (5FUP4) are described in the following section.

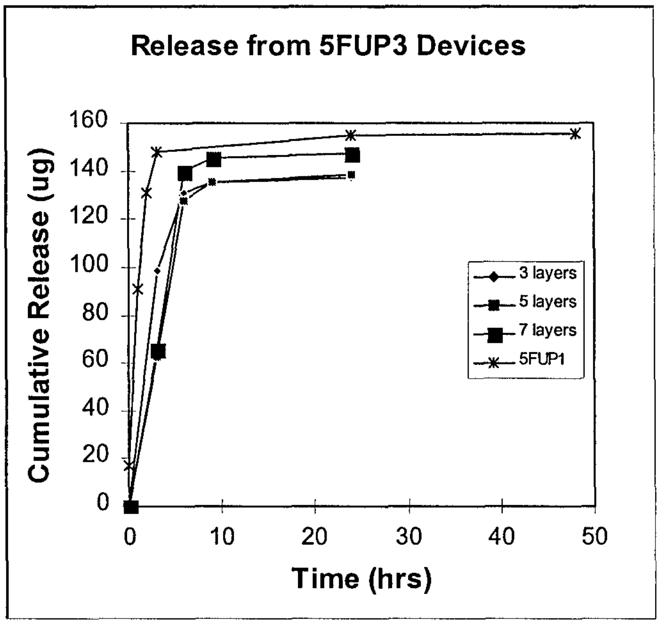


Figure 13. Cumulative release of 5-FU from 5FUP3 devices.

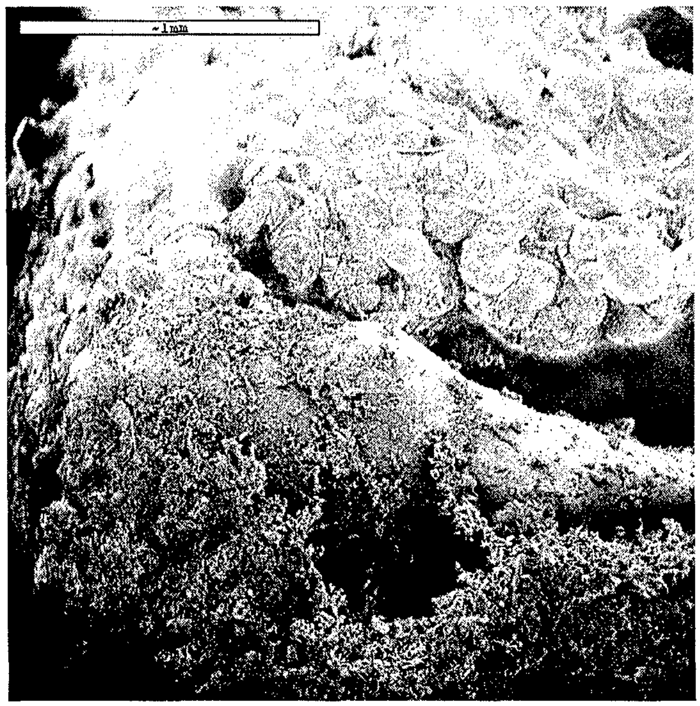


Figure 14. SEM of a 5FUP3 device taken from the side at an angle (40X, 57° tilt). Shown in the upper right quadrant is the P(FAD:SA) lid. Poor bonding to the PLGA walls caused the formation of a large opening shown in the right midsection of the micrograph.

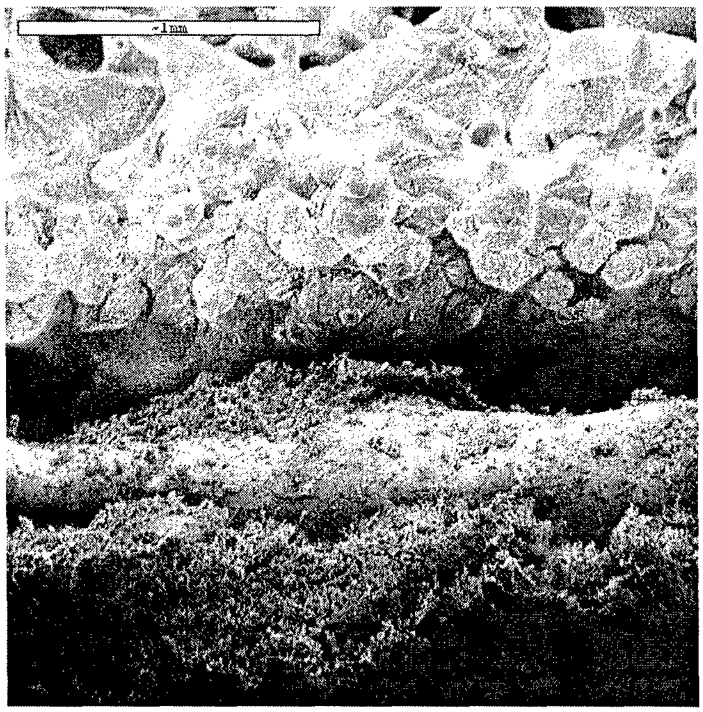


Figure 15. **SEM of a 5FUP3 device taken from a side view (40X). P(FAD:SA) layer is shown on the top and the PLGA walls on the bottom. A small gap between the lid and the wall is observed on the right.**

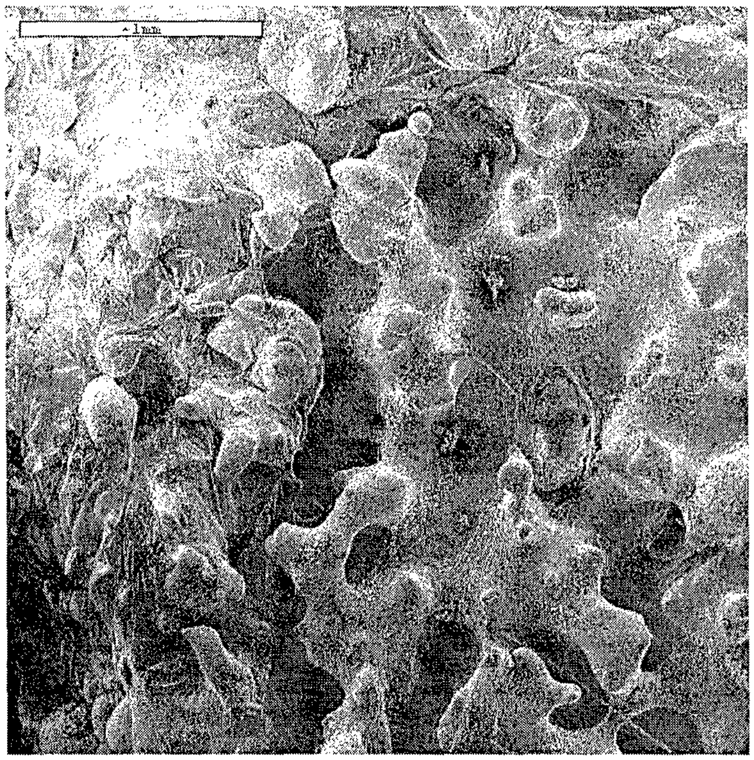


Figure 16. **SEM (30X) taken from the top surface of the P(FAD:SA) lid layers in 5FU3 devices (170μm line spacing, double printing of chloroform).**

5-FU Prototype 4 (5FUP4): Several fabrication parameters were modified to enhance the structure of the 5-FU prototype devices as described above. There are three regions from where the 5-FU could have released prematurely from the 5FUP3 devices: a) through the pores in the PLGA walls, b) through the pores in the P(FAD:SA) lid, and/or c) through the interfacial defect between PLGA walls and the P(FAD:SA) lid. The process parameters used for building 5FUP4 were designed to minimize, if not eliminate, any premature release due to each of the three possible causes. The PLGA walls were built with twice as much chloroform binder as the 5FUP3 devices by using a double print technique. Figure 17 shows the microstructure of the resulting walls that are relatively defect-free. Top surface of PLGA walls were pre-saturated with chloroform prior to constructing the P(FAD:SA) layers in an effort to facilitate the bonding between two polymer systems. The line spacing used for constructing the P(FAD:SA) lid was reduced to 85μm to effectively increase the binder amount for the lid layers. Resulting P(FAD:SA) lid shows a significant improvement in the microstructure from that of 5FUP3 devices as illustrated in Figure 18 compared to Figure 16.

Release data from 5FUP4 devices is summarized in Figure 19. Unlike the 5FUP3 devices, 5FUP4 devices exhibited a significant lag time before any release of 5-FU. Figure 20 shows the initial stage of the 5FUP4 release study and a consistent lag time of ~ 8 hours. This is in contrast to the previous prototype devices which showed an immediate release of 5-FU regardless of the release layer thickness. Presence of the initial lag suggests the elimination of the structural defects that were present in the earlier prototype devices. This is in accordance with the improved microstructure as shown in Figures 17 and 18. The overall release rates of the devices, however, still present a drug release mechanism that is not obvious. Examination of the release rate on the eroding lid thickness reveals a counterintuitive correlation. Figure 19 shows that the prototype devices with thicker erosion lids released 5-FU faster than the ones with thinner erosion lids. One may postulate that the 5-FU is not releasing through the top lids, but through the interface between PLGA walls and the P(FAD:SA) lids to explain this phenomenon. Figure 21 illustrates effect of the P(FAD:SA) layer thickness on the interface microstructure. Shrinkage of the printed regions during the layer by layer build processes is known to cause distortions in the resulting components. The extent of warping effect initially increases as the number of layers increases. The net effect in the prototype devices would be the decrease in the diffusion path for 5-FU with increasing number of lid layers in the case of imperfect PLGA:P(FAD:SA) interfaces.

It would seem then that one needs to eliminate warping effects to achieve a predictable drug release from the devices. Accordingly, the prototype design was modified to accommodate better interfacial bonding and eliminate the adverse effects from the warping of the lids. Details of the design modification and results are described in the following section.

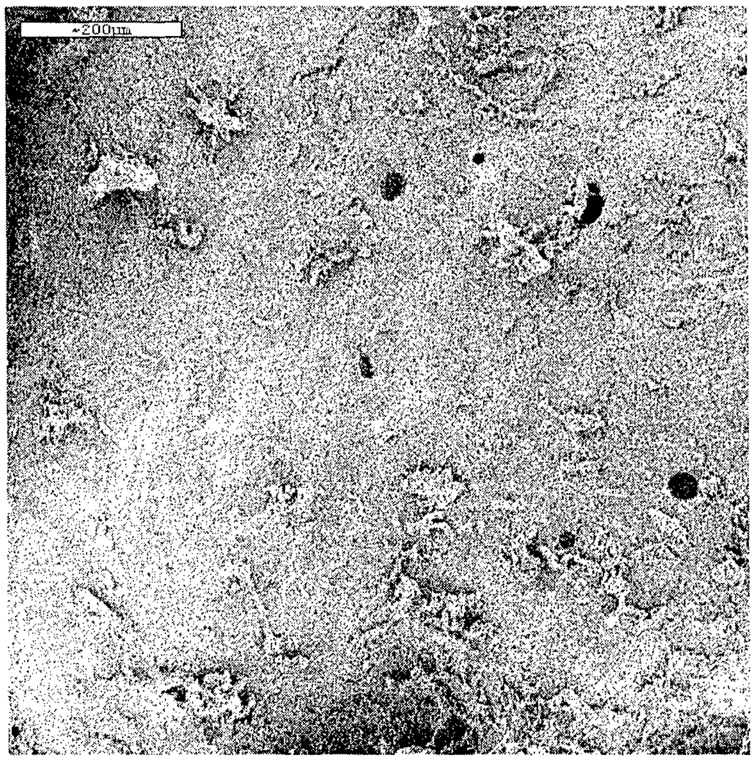


Figure 17. SEM (100X) taken from side wall of a 5FUP4 device (170μm line spacing, double printing of chloroform). These PLGA walls were relatively defect-free.

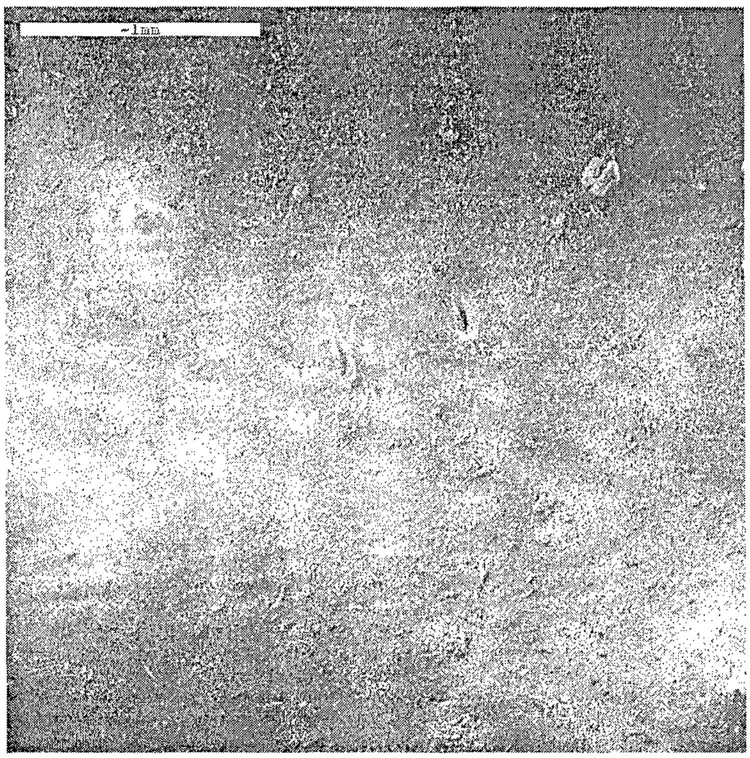


Figure 18. **SEM (30X) taken from a 5FUP4 device's top layer. The lid layer appears to be smooth and pore-free.**

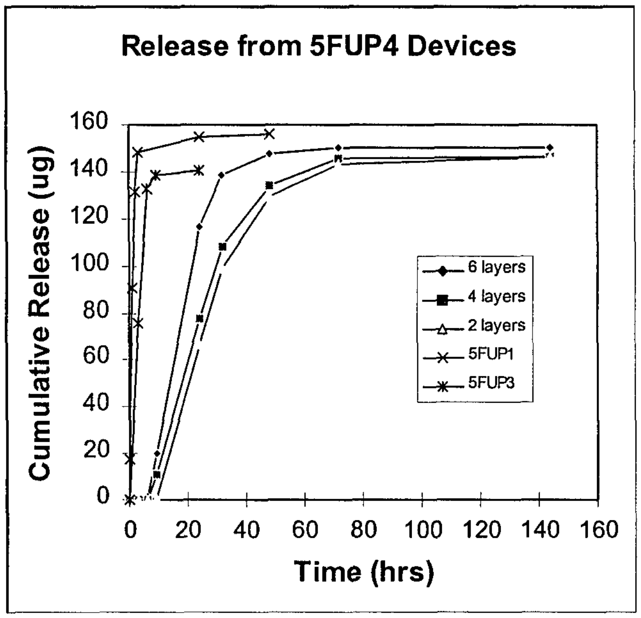


Figure 19. **Cumulative release of 5-FU from 5FUP4 devices with different number of lid layers (n=4).**

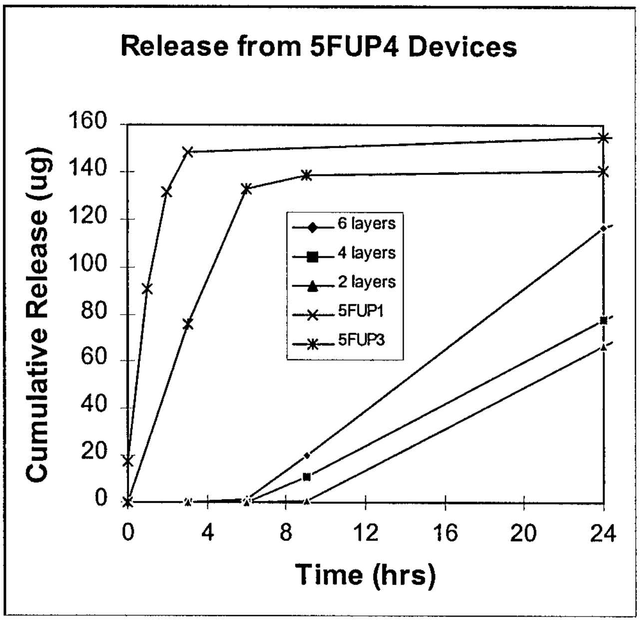


Figure 20. Initial stage of the 5FU release data from 5FUP4 implants exhibiting a lag time of ~ 8 hours.

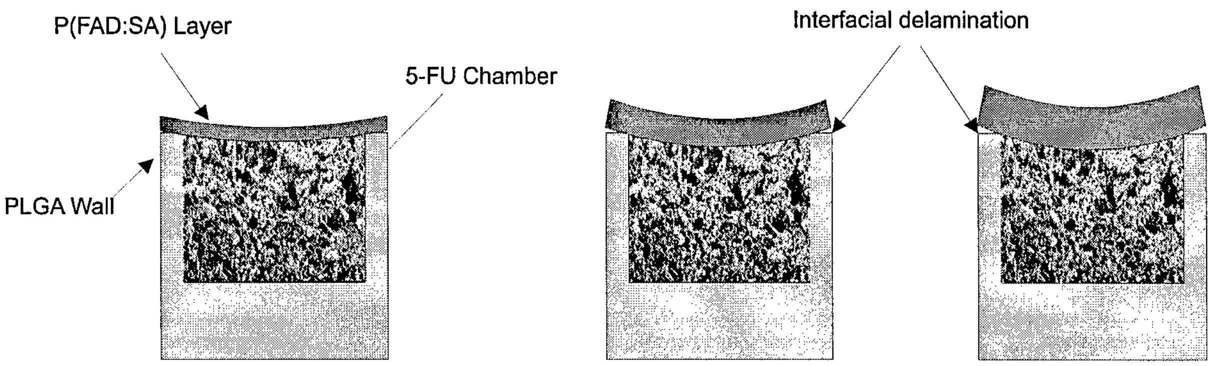


Figure 21. Schematic of the prototype devices and the effect of warping on the diffusion paths for 5-FU.

5-FU Prototype 5 (5FUP5): Thickness of the side walls in the 5FUP5 were increased to 8 lines in contrast to that of the 4 lines in the previous prototype devices. The rationale behind this design was to increase the surface area of the PLGA:P(FAD:SA) interface. This would increase the integrity of the interfacial bonding and decrease the chance of the erosion layer warping. Release of 5-FU from these devices is summarized in Figure 22. The release profiles of these devices are highly dependent on the thickness of the lids. All three sets of devices show constant rate of release for the most part and the release rates are distinctively different from each other. Approximate release rates of the three prototype sets are summarized in Table 5. Examination of the initial stage also exhibits a significant lag time prior to the zero-order release as shown in Figure 23. This lag time is in the order of 12 to 18 hours in the 5FUP5 devices with 4 layers of P(FAD:SA) lid. Sustained release of 5-FU for more than 160 hours from these devices suggests that most of the defects associated with the earlier prototype designs have been eliminated.

Another interesting observation from this set of prototype devices is the linearity of the drug release profile. This type of release characteristics is consistent with other *"capsule-type"* polymeric delivery devices.[14] In these *capsule-type devices*, a constant drug concentration gradient across the diffusion layer results in a constant rate of drug release. The release rate from these devices is dependent on the thickness of the diffusion layer, which is consistent with our observations summarized in Table 5. Measurement of the dimensions of the devices prior to the release study also confirmed a linear relationship between the number of constructed lid layers and the total device thickness as illustrated in Figure 24. The erosion rate of the P(FAD:SA) layers may not have been high enough to affect the release rate of 5FUP5 devices. In other words, all of the 5-FU from the devices has diffused through the P(FAD:SA) layer before the lid layers had eroded to a point of rupture to cause a burst of 5-FU. Physical design of the devices would have to be modified to accommodate the pulsatile release of 5-FU. One such design would include a device where separate drug chambers would be exposed to the dissolution medium in a serial fashion, rather than a parallel fashion as originally proposed in this study.

Table 5. Drug release rate from 5-FU Prototype 5 (5FUP5) devices with different lid thickness.

Number of P(FAD:SA) layers (170μm each)	Release rate (μg/hour)
2	4 μg/hour
3	1.5 ~ 2.3 μg/hour
4	0.9 μg/hour

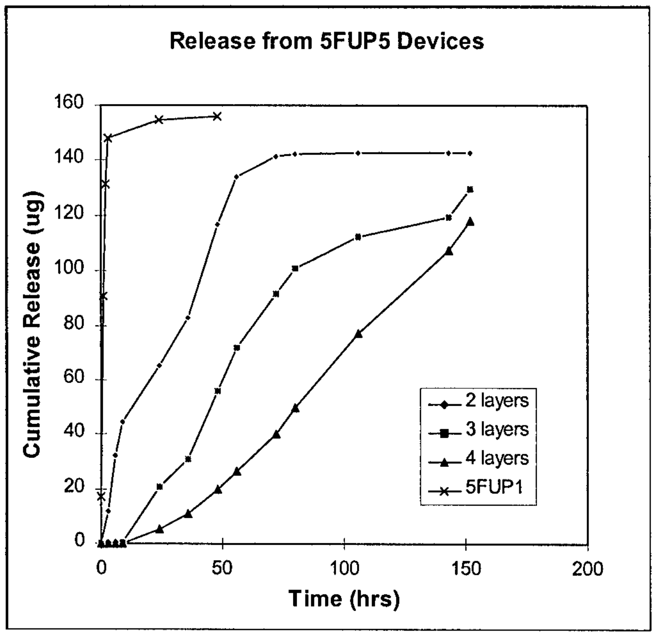


Figure 22. Cumulative release of 5-FU from 5FUP5 devices (n=4).

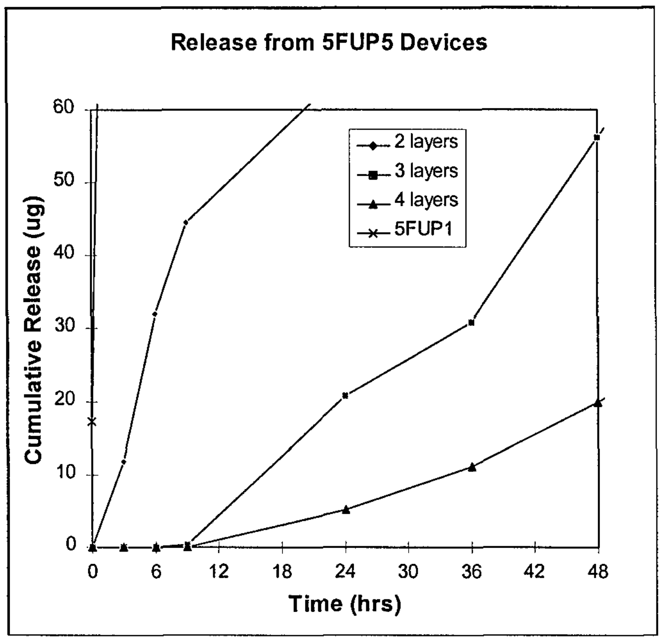


Figure 23. Initial stage of drug release from 5FUP5 devices (n=4).

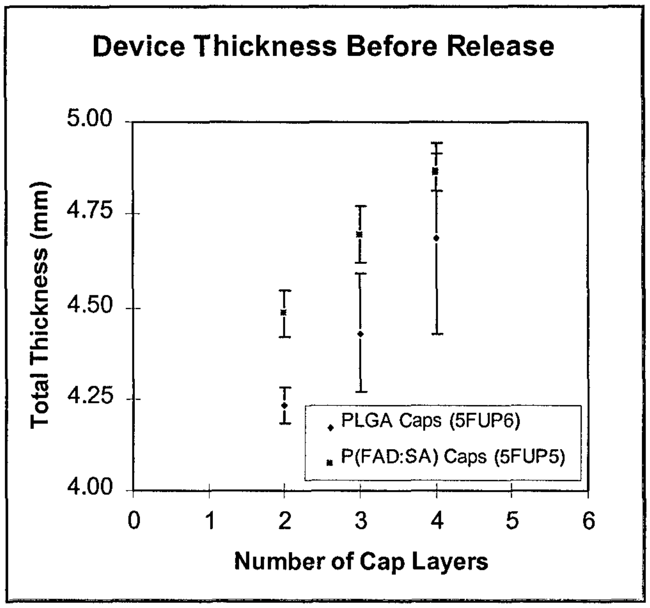


Figure 24. Thickness of 5FUP5 and 5FUP6 prototype devices as a function of number of lid layers.

5-FU Prototype 6 (5FUP6): 5FUP6 devices were fabricated entirely of PLGA, including the top lids. The intention was to test the extent of 5-FU diffusion through PLGA walls. Results from earlier prototypes indicated that the release of 5-FU is occurring in the P(FAD:SA) layers or at the P(FAD:SA) and PLGA interfaces. The release rate dependency on the P(FAD:SA) layer thickness from the 5FUP5 devices is direct evidence supporting this theory. Thus the devices fabricated entirely of PLGA were expected to have very little 5-FU release until physical degradation of the device matrix would take place. Release study of 5FUP6 devices has shown an interesting result as shown in Figure 25. 5-FU released immediately from 5FUP6 devices and no apparent difference was observed between devices of varying lid thickness.

Microstructure of the devices was examined and revealed interesting characteristics around the PLGA lid. Figure 26 is a micrograph of the PLGA lid surface which exhibits many large interconnected pores. Side and bottom faces of the 5FUP6 devices showed a very dense structure, as shown in Figure 27. This deviation in the microstructure can be traced to the conditions at which respective regions were fabricated. Every fabrication parameter, including binder flow rate, printing scan speed, line spacing, and material used were kept identical. The only significant difference between the fabrication of the side walls versus the construction of the lid layers is the architecture of the material beneath the respective region during fabrication. Bottom layer of the devices and the side walls are always constructed upon solid, or semi-solid substrates. When an overhang is fabricated on a cushion of loose powder, the powder-binder interaction may be significantly different from the case of solid substrates. This deviation in the binder interaction mechanism may be the cause of the porous microstructure of the 5FUP6 device lids.

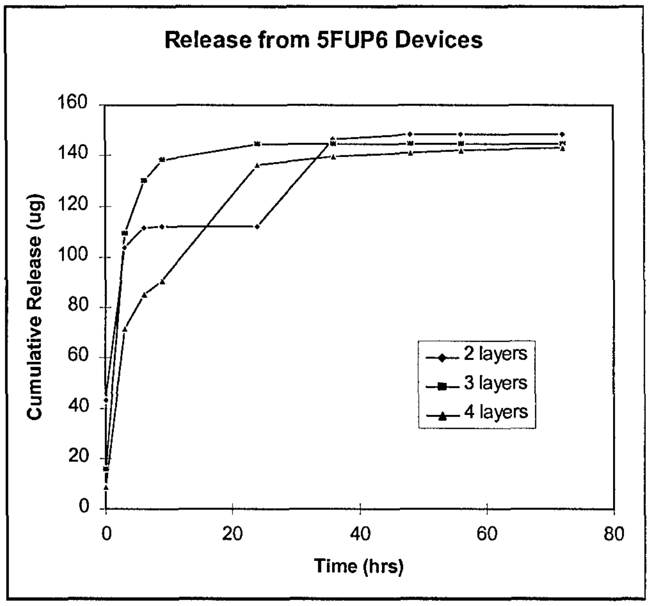


Figure 25. Cumulative release of 5-FU from 5FUP6 devices (n=4).

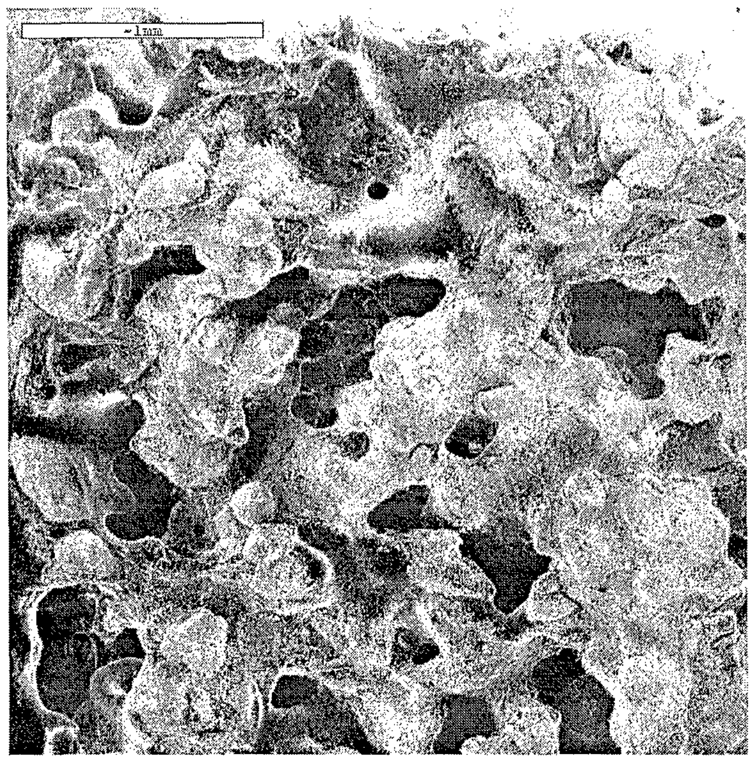


Figure 26. **SEM (30X) taken from the top surface of a 5FUP6 device's PLGA lid. Interconnected pores are evident from the micrograph.**

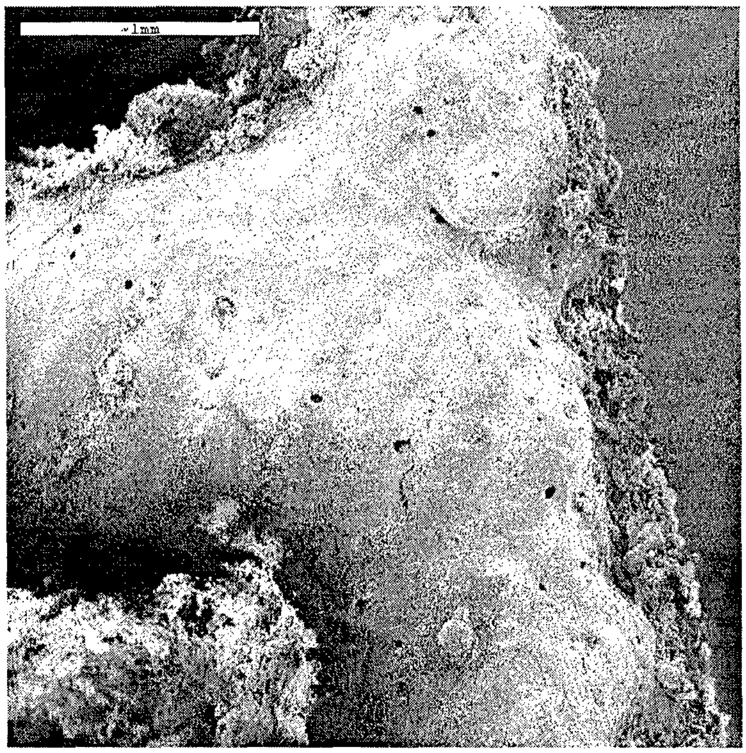


Figure 27. SEM (30X) taken from a 5FUP6 device's dense side walls.

2.2.3. Conclusions

Iterations of prototype device fabrication and testing yielded much valuable information that can be utilized for any drug delivery systems in general. Microstructure analysis indicated that the PLGA walls fabricated in this study using TheriForm™ process were very dense and do not have interconnected pores. The PLGA films degraded in a relatively slow fashion and, therefore, were selected for fabricating the walls of the implant chambers containing 5-FU. Release profiles of the prototype devices have shown the effectiveness of these PLGA walls as an impermeable barrier for the 5-FU diffusion. 5FUP5 devices in particular, have demonstrated that the release rate of 5-FU can be controlled by merely changing the number of P(FAD:SA) lid layers as illustrated in Figure 22. Microstructure of the P(FAD:SA) lid layers was found to be highly dependent on fabrication parameters. Significant improvement in the layer microstructure was achieved by optimizing the binder amount.

One of the most important results of this study was the confirmation of the processing-microstructure relationship. Earlier iterations of the prototype design and fabrication have demonstrated that the structural imperfections caused by the construction process may lead to premature release of drug from the chambers. One area that is the most prone to develop a structural imperfection was determined to be the interface between the side walls and the top lids. Earlier prototypes, 5FUP3 devices in particular, have shown dose dumping due to improper bonding between the two regions. Simple modifications in the process to pre-saturate the side walls apparently enhanced the bonding between walls and the lid layers. Further improvements in the device performance were observed after increasing the surface area of the interfacial region.

Several different modes of 5-FU release were observed from the prototype devices. Open architecture of the 5FUP1 devices exhibited an immediate pulse of 5-FU within the 3 hours of the release study. This data clearly demonstrates that pulsatile release may be achieved if the drug-containing chambers are capped with lids of proper material and thickness. 5FUP3 devices showed an immediate release similar to that of 5FUP1. The 5FUP3 device design, however, would be preferred for providing the first pulsatile release at time zero compared to an open chamber since the loosely bonded lid layer of 5FUP3 will prevent the unbound PLGA powder and 5-FU from spilling out of the chamber. It is only when the device is in contact with the liquid medium that the 5-FU molecules start to exit the chambers.

Different lag times were demonstrated by the 5FUP4 and 5FUP5 devices, ~ 8 hours and ~ 12 hours, respectively. In addition, 5FUP5 devices demonstrated a clear relationship between the lid layer thickness and the 5-FU release rate. Constant rates of release were observed in these devices which is consistent with the *capsule type* drug delivery systems.[14] This observation suggests that the material system chosen for this study P(FAD:SA) does not erode fast enough to cause failure. Instead, pore channels developed in the layer allowed the diffusion of 5-FU, resulting in a zero order release. This prototype design can be utilized for sustained release of pharmaceutical agents at a constant rate. Since the rate is dependent on the thickness of the lid layer, one can tailor the design of the devices to match the required dosage level. Pulsatile release of 5-FU, however, requires the polymeric lid to permit very little diffusion while rapidly eroding away to cause a catastrophic failure to cause bursts. It is envisaged that this mode of release may be achieved by using other rapidly eroding polymers. Alternatively, a modification in the prototype design may accommodate a sequential exposure of release layers to the dissolution medium. For example, stacks of the current prototypes would result in such a configuration.

Note: Data for the 5-FU implant fabrication and testing is recorded in Therics' laboratory notebook 003, pages 9-86.

2.3. Diclofenac implant fabrication and testing

2.3.1. Experimental

2.3.1.1. Binder selection

Polyesters, such as poly lactic acid (PLA) and poly lactic-co-glycolic acid (PLGA), were chosen as the excipients for the implant because polyesters are the only class of bioresorbable polymers with a long history of FDA-approved products. The most well known use of polyesters is for biodegradable sutures, which have had an excellent safety record. TheriForm™ fabrication of implants involves deposition of a binder solution at desired points on successive layers of polyester powder spread in the powder bed. This binder solution may or may not include polyester and/or active agents such as diclofenac sodium (diclofenac, DC) or 5-FU. The choice of solvents for polyesters is limited to chloroform, dichloromethane, and hexafluoroisopropanol (HFIP). HFIP was eliminated as a candidate owing to its extremely toxic side effects. Dichloromethane was found to be incompatible with the TheriForm™ technology when using polymer in the binder because of the high volatility of dichloromethane, which evaporates quickly thereby clogging the nozzle with the polymer. Thus, chloroform, with its lower volatility, was determined to be the best solvent for the polyester polymers. As chloroform is deposited through the nozzle onto the powder bed, it dissolves the superficial layers of the polyester particles in the powder bed. As the chloroform evaporates, it binds the particles where it was deposited.

A uniform distribution of diclofenac in the implant may be achieved by dispensing it as part of the binder solution. However, diclofenac is not adequately soluble in chloroform to provide the desired drug loading. Since diclofenac is most soluble in methanol, but chloroform is required to dissolve the polymer, the solubility of diclofenac in different ratios of methanol and chloroform was investigated. In addition, the ability of these solvent combinations to dissolve polyesters was examined.

2.3.1.2. Filter study

A 20 μg/ml standard stock solution of diclofenac in 70 mM sodium phosphate buffer solution (pH 7.32) was used to determine if diclofenac was binding to the Teflon filters used in the controlled release studies. The concentration of the diclofenac standard stock solution was verified using UV-VIS spectroscopy. Ten ml of the stock solution was drawn into a 10-ml syringe (Becton Dickinson). A Teflon filter (3 Spartan-T, 0.2 μm, 25 mm, lot: BIFZ115) was placed on the end of the syringe to filter the solution into a 20-ml glass scintillation vial. Two additional 10-ml aliquots were filtered with the same filter and collected into separate vials. This process was repeated with two additional filters. The samples were stored at 2°C until analysis by HPLC.

2.3.1.3. Diclofenac implant fabrication and drug release

The diclofenac component of the implant was approximately a 5.6 mm x 5.6 mm x 1.5 mm section made with PLGA (50:50) 50 KDa (Boehringer Ingelheim, RG504, Lot 34015), polymer and contained a minimum of 1.12 mg of diclofenac (the amount needed to release 80 μg/day for 14 days). Although, there were variations between the prototype, as described below in more detail. The polymer was placed in the powder bed and the diclofenac is dispensed as part of the binder solution (24 mg/ml of diclofenac in a 1:4 methanol: chloroform mixture) through the nozzle of the TheriForm™ machine during fabrication. Table 6 lists the printing parameters used to fabricate the different prototypes.

Diclofenac (DC) Prototypes: Prototypes 1 and 2 were fabricated as 1-cm disks. Prototype 1 consisted of only a diclofenac region, whereas prototype 2 also had an inert portion to represent the 5-FU part of the device; this also prevented release of diclofenac from one face of the disk. Prototypes 3, 4, and 5 were cubes containing diclofenac made to simulate the entire intraocular implant. Thus, the bottom section in these prototypes consisted of inert polymer to represent the 5-FU portion of the actual implant, the middle section was the diclofenac-polymer portion, and the thin top section was a polymer or polymer-NaCl cap used to reduce the initial burst of diclofenac. Double sided tape and stilts were used to prevent the cubes from moving during fabrication. The diclofenac portion was double printed (binder was deposited twice on each layer) in order to achieve the proper loading of diclofenac.

DC Prototype 1: The first batch of diclofenac-containing implant prototypes was made using a template with 1 cm diameter holes. Although this template does not represent the desired design, it was used to fabricate prototypes to get some preliminary information on the release rates of diclofenac. The prototypes were made with PLGA (50:50) 50 KDa in the powder bed and 24 mg/ml of diclofenac in a 1:4 mixture of methanol:chloroform was dispensed through the nozzle to create homogeneous prototypes.

DC Prototype 2: A second batch of diclofenac prototypes was made similar to the first, except these prototypes were approximately twice as thick. The extra thickness was plain PLGA without diclofenac to better simulate the design of the final device (i.e., diclofenac was only able to release through one face of the device).

DC Prototype 3: A 5.6 x 5.6 mm mask was used to fabricate this batch of devices to the proper *in vivo* size, containing the medicinal dosage of diclofenac. Release through the one face was prevented, as before, by fabricating 12 layers using 1:1 blend of PLGA (50:50) 50 KDa and L-PLA (Birmingham Polymers, Lactel, Lot D96104) to represent the 5-FU portion of the final design. The middle diclofenac section was made with PLGA (50:50) 50 KDa in the powder bed and diclofenac was deposited through the nozzle. This batch was divided into 4 groups, each with a different number of top layers (from 1 to 4) coating the other face of the device that was formerly exposed. These caps were introduced to prevent the initial burst of diclofenac release. PLGA (50:50) 50 KDa was used to fabricated these caps.

Table 6. Fabrication parameters for the diclofenac portion of device.

Prototype	Flow Rate (ml/min)	Print Speed (m/s)	Line Spacing (μm)	Layer Height (μm)	Number of Layers
1	1.2	1.0	158	200	12
2	1.2	1.0	158	200	12
3	1.2	1.5	155	200	6
4	1.2	1.5	155	200	7
5	1.2	1.5	155	200	7

DC Prototype 4: Prototype 4 was similar to prototype 3 except that the caps were 2 layers thick and contained 30 % w/w NaCl (Fisher Scientific, Lot 975737). The preliminary hypothesis was that once immersed in an aqueous environment, the NaCl would dissolve leaving behind pores in the caps, which increased the surface area available for diclofenac diffusion.

DC Prototype 5: Owing to the results of prototype 4, prototype 5 was fabricated with 35% NaCl in the diclofenac portion of the device to ensure that the diclofenac channels through the polymer phase would be adequately interconnected according to percolation theory. The extent of interconnection was important to ensure that significant amounts of the diclofenac were not trapped in the polymer phase. The caps in prototype 5 did not contain NaCl, similar to prototype 3. Three variations of prototype 5 were fabricated and tested. Prototype 5a was similar to prototype 3 with the exceptions mentioned above (35% NaCl/65% PLGA (50:50) 50 KDa in the diclofenac section and no NaCl in the cap). Prototype 5b was similar to prototype 5a except that the diclofenac section contained 35% NaCl/35% PLGA (50:50) 50 KDa/30% PLGA (50:50) 4.5 KDa (Boehringer Ingelheim, RG501, Lot 56017). Prototype 5c was similar to prototype 5a except that the caps were fabricated with PLGA (50:50) 4.5 KDa instead of PLGA (50:50) 50 KDa.

Drug release studies: The diclofenac devices were placed in 70 mM PBS at 37°C under static conditions. The solution was changed at regular intervals and the amount of diclofenac released into PBS was determined by HPLC.

2.3.1.4. Residual solvent removal

Prototype 1 devices were held in liquid CO_2 at 4.4°C and 800 psi for 5 minutes, then vented for 30-40 minutes. Devices from prototype batches 3 and 4 were treated the same way except that they were exposed to liquid CO_2 for 30 or 60 minutes before venting for 30-40 minutes.

2.3.2. Results and Discussion

2.3.2.1. Binder selection

A maximum concentration of 34 mg/ml of diclofenac was achieved in a 1:1 methanol: chloroform solution, and 24 mg/ml of diclofenac in 1:4 methanol:chloroform. Choosing the best binder was a balance between having enough chloroform to dissolve the polymer in the powder bed and having enough methanol to dissolve a large amount of diclofenac. 100% chloroform has been used with polyesters in the past and believe that 80% chloroform will be adequate but 50% chloroform will probably not be enough. Thus, 24 mg/ml diclofenac in a 1:4 mixture of methanol:chloroform was used with the printing parameters listed in Table 6. By double printing each layer, i.e., by depositing binder twice on each layer, the desired amount of diclofenac in the device was achieved, while remaining within the device size specifications.

2.3.2.2. Filter study

The filter study was conducted to determine if diclofenac was binding to the filters used for in the controlled release study. The concentration of the diclofenac stock solution was determined to be 19.02 μg/ml. The average amount of diclofenac lost from the first pass of the filter was 5.5 ± 0.3%, 3.3 ± 0.1% from the second use, and 3.3 ± 0.3% from the third pass, see Table 7. This study indicated that the filters may retain a small but reproducible amount of diclofenac. In the future, the filters may be pre-wet with diclofenac solution or a different type of filter may be used.

2.3.2.3. Diclofenac implant fabrication and drug release

DC Prototype 1: The resulting 22 prototypes averaged 151 ± 10 mg in weight, 1.8 ± 0.1 mm in thickness, 1.16 ± 0.06 cm in diameter (n = 22), and contained an average of 7.3 ± 0.2 mg (n = 4) of diclofenac. Six of these prototypes were exposed to liquid CO_2 to remove residual chloroform and determine if this process affects the diclofenac content. The weight of these 6 prototypes had decreased 17 ± 4% after liquid CO_2 drying. However, the amount of diclofenac in the disks exposed to liquid CO_2 was 7.2 ± 0.2 mg (n = 3), which was the same as disks not subjected to liquid CO_2. The amount of residual chloroform in disks exposed to liquid CO_2 was 1.9 ± 0.3% (n = 3), compared to 5.5 ± 0.3% (n = 3) for control devices not exposed to liquid CO_2. Thus, exposure to liquid CO_2 under the these conditions reduced the amount of chloroform by 65% but did not affect diclofenac content. Longer exposure to liquid CO_2 may further reduce this amount of residual chloroform since the extraction process is diffusion controlled.

Table 7. Binding of diclofenac to Teflon filters.

Filter	First Pass Diclofenac Loss (%)	Second Pass Diclofenac Loss (%)	Third Pass Diclofenac Loss (%)
A	5.5	3.2	3.6
B	5.5	3.4	3.3
C	5.1	3.2	3.0
Average	5.4 ± 0.3	3.3 ± 0.1	3.3 ± 0.3

DC Prototype 2: These prototypes averaged 304 ± 7 mg in weight, 4.26 ± 0.08 mm in thickness, and 1.03 ± 0.01 mm in diameter (n = 22). The initial amount of diclofenac in these prototypes was analytically determined to be 4.9 ± 0.6 mg (n = 4). Two sets of disks from this batch underwent a release study in phosphate buffered saline (PBS). Figure 28 shows the average cumulative release of diclofenac into PBS at 37°C under static conditions (2x n = 3). Nearly half of the drug was released within the first 5 days. Over 16 days, the disks released 61% of the incorporated diclofenac, with 40% released within the first 5 days. The rate of diclofenac release is shown in Figure 29. The amount of diclofenac released was initially high then fell below the desired release rate. To decrease this initial burst of diclofenac release, the next prototype was fabricated with the large face of the device coated with PLGA (50:50) 50 KDa.

DC Prototype 3: These prototype 3 devices were determined to contain 1.23 ± 0.02 mg of diclofenac (n = 14), and were 4.3 ± 1.7 mm high, and weighed 89 ± 5 mg (n = 77). The PLGA coating was expected to form an incomplete barrier to diffusion. The hypothesis was that the initial burst release of diclofenac could be reduced by decreasing the surface area of the diclofenac-containing implant available for diffusion, i.e., regions that directly come in contact with the aqueous media during drug release studies. Pores in this barrier were expected to allow release of small amounts of diclofenac initially, with increasing amounts released as the barrier cap degraded.

Figure 30 shows the release of diclofenac from disks with 1 to 4 layers of PLGA coating from the DC prototype 3 batch (n = 3). The release of diclofenac from all of these devices was substantially decreased from the previous uncapped batch. After 4 days, approximately 0.1 mg had been released, which is 8% of the total diclofenac content. The number of layers did not significantly affect amount of diclofenac released over the first several days. This suggests that the PLGA coating did form a relatively complete barrier to the diclofenac and that most of the release must be occurring from the sides. The rate of diclofenac release was approximately 66 mg/day the first day and then dropped to less than 15 mg/day for the next 6 days before increasing to 25-70 mg/day for days 7 -10, as shown in Figure 31. Day 13 had a significant increase in diclofenac release rate, possibly owing to the polymer degradation. The rate of diclofenac release was lower than the desired release; previously the release rate was too fast, thus, release rates that cover both extremes were achieved. The last two days were closer to the desired release rate of 80 μg/day.

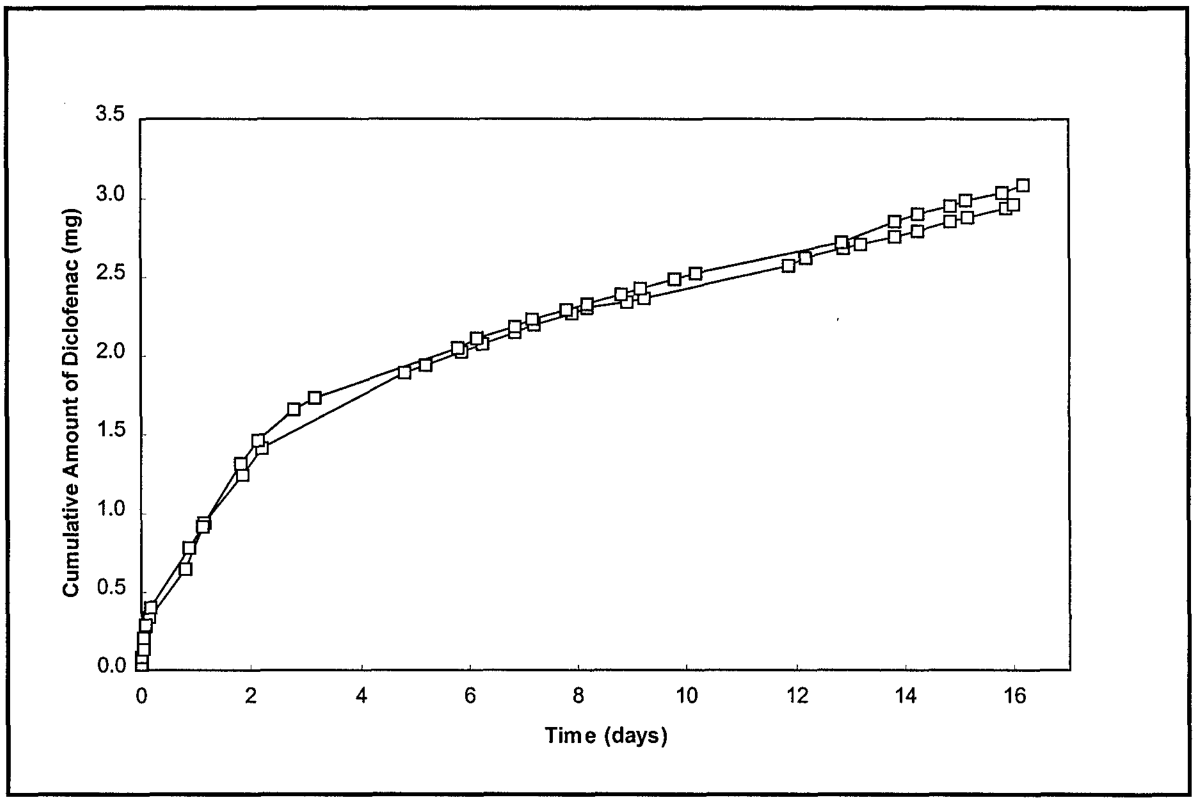


Figure 28. **Cumulative release of diclofenac from DC Prototype 2 disks. Two separate controlled release studies were performed with disks from this batch.**

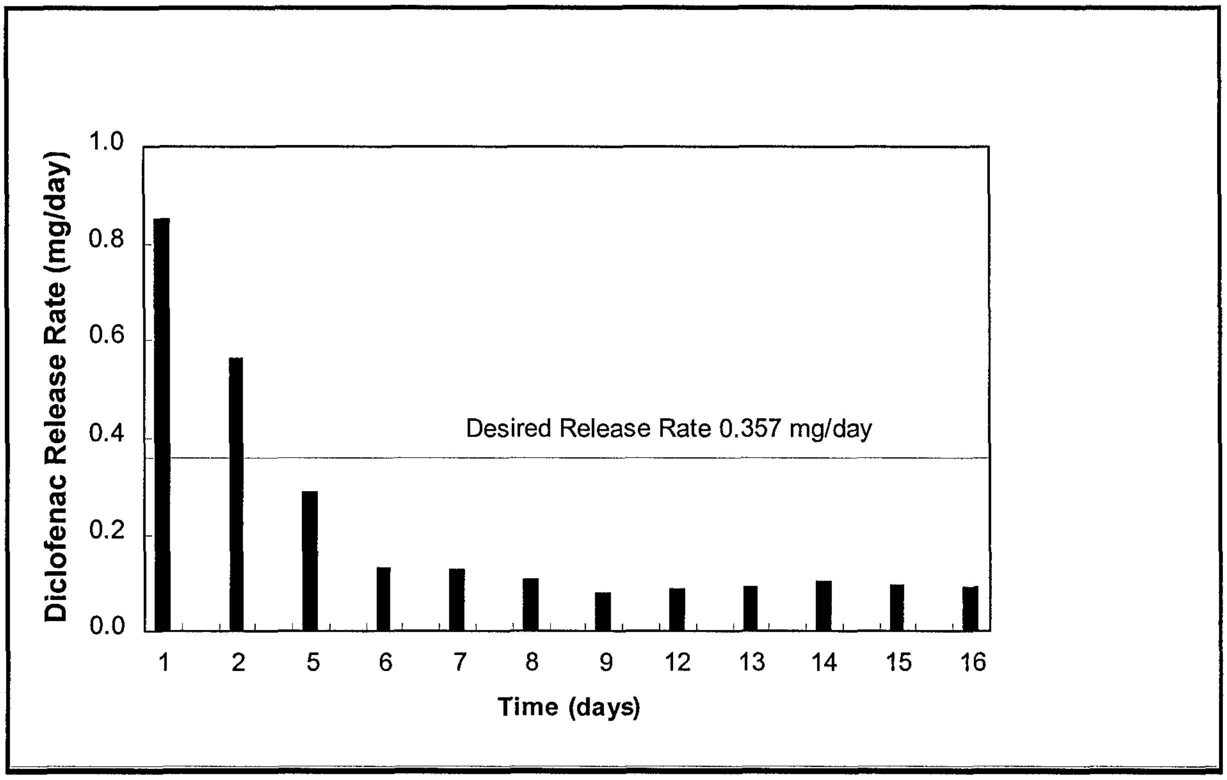


Figure 29. **Diclofenac release rate from DC Prototype 2 disks. The desired release rate of diclofenac from these disks was calculated using the percentage of diclofenac in this batch compared to the actual implant.**

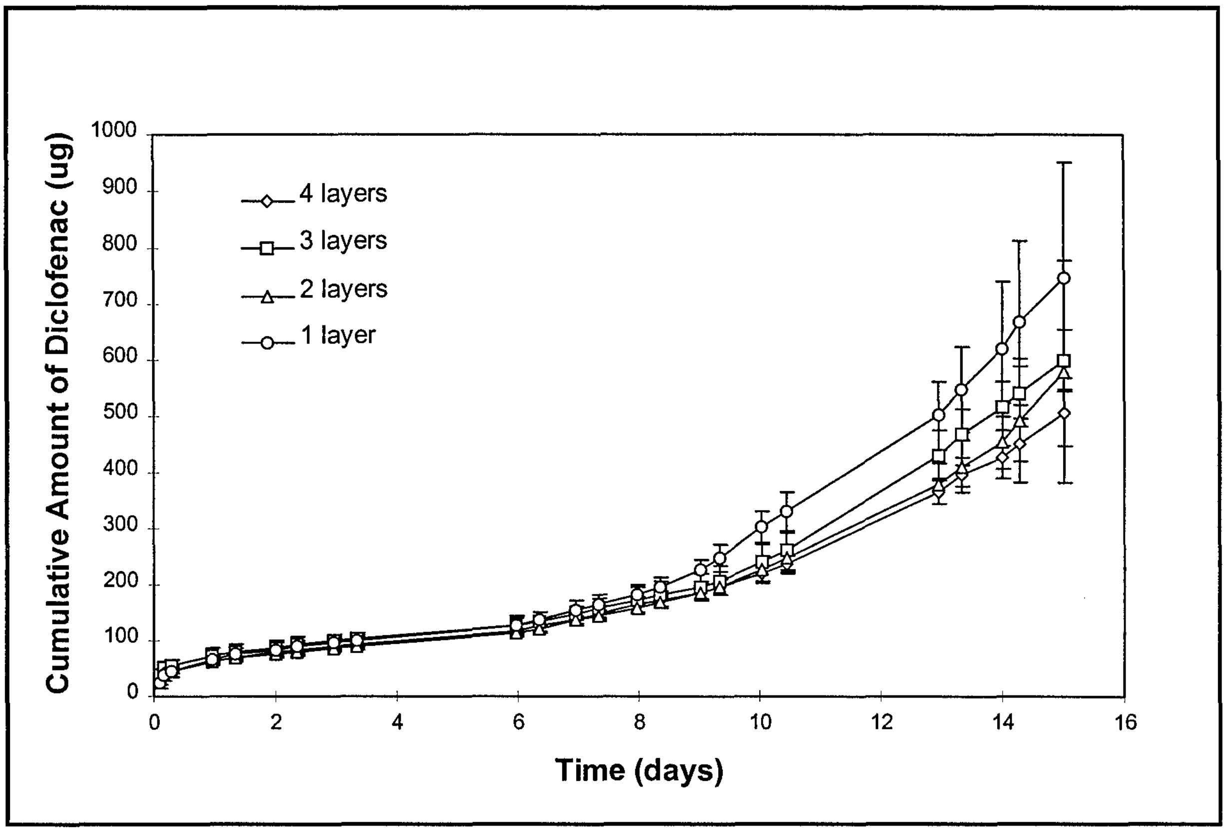


Figure 30. **Cumulative release of diclofenac from DC Prototype 3 cubes. These cubes have 1-4 layers thick caps.**

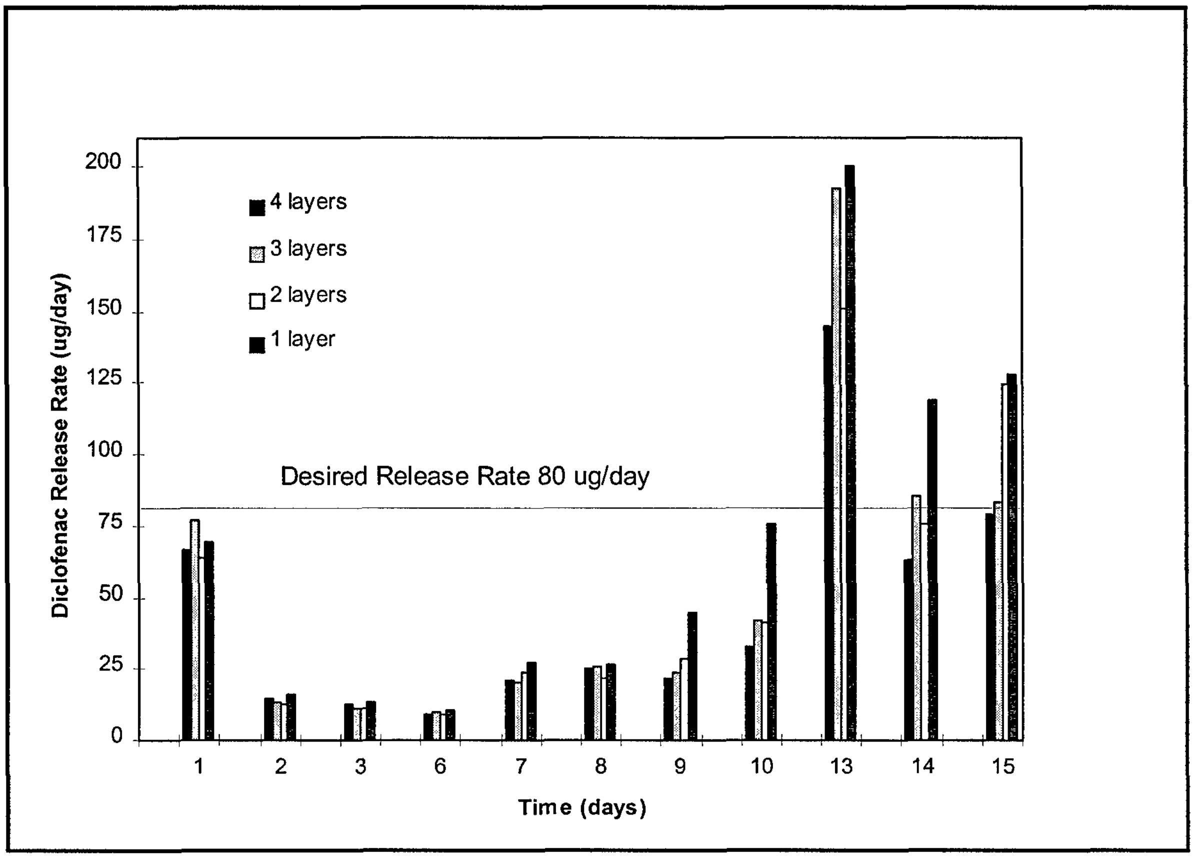


Figure 31. **Rate of diclofenac release from DC Prototype 3 cubes. The desired release rate is 80 μg/day.**

DC Prototype 4: Prototype 4 cubes weighed 59.4 ± 0.6 mg, had a height of 2.4 ± 0.1 mm, a length of 5.6 ± 0.2 mm, a width of 5.4 ± 0.1 mm, and the middle diclofenac section was 1.2 + 0.1 mm high (n = 24). Controlled release studies of these devices were conducted in water as well as PBS. The first 7 days of release were identical but at later times very little diclofenac was released into water, as shown in Figure 32 (n = 3). Approximately 140 μg was released the first day. For the next 13 days, less than 30 μg was released a day. On days 14, 15, and 16, an average of 56, 85, and 117 μg/day of diclofenac was released into PBS, as shown in Figure 33.

This batch was similar to prototype 3 except that the caps were 2 layers thick and contained 30% NaCl. The NaCl was used to create holes in the cap in order to increase the rate of diclofenac release by increasing the surface area available for diffusion. These devices had a larger initial diclofenac burst on the first day than prototype 3 but did not increase the rate or amount released on the following days as anticipated. Thus, the release of diclofenac was not merely limited by the surface area available for diffusion but rather by the diclofenac loading, which influences the tortuosity of the interconnected diclofenac channels and therefore the random walk path length necessary for the diclofenac molecules to traverse in order to be released into the PBS solution.

The lower amount of diclofenac released into water versus PBS may possibly be attributed to a drop in diclofenac solubility as the polymer degrades making the water more acidic, since water is a poor buffer. In PBS, diclofenac is ionized and therefore soluble, while in acidic water diclofenac may be neutral and therefore less soluble (the pKa of diclofenac in water is 4).

DC Prototype 5: The dimensions of the three prototype 5 groups of cubes are listed in Table 8 (n = 24). Figures 34 and 35 show the cumulative release and release rate of diclofenac from prototype 5 cubes, respectively (n = 3). The release rate from prototype 5b cubes is right on the mark at 80 μg/day. Thus, the addition of salt to the diclofenac portion to interconnect the diclofenac channels and the addition of the faster degrading PLGA (50:50) 4.5 KDa were successful in obtaining the desired release rate.

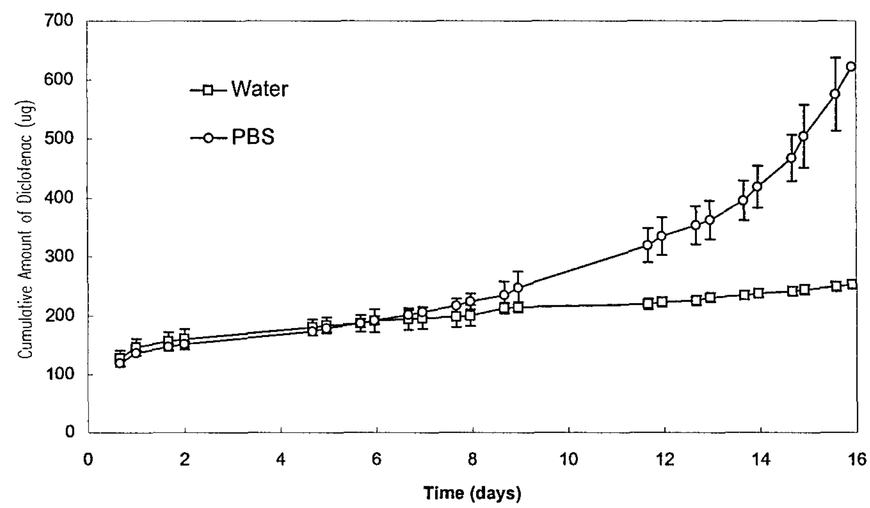


Figure 32. **Cumulative release of diclofenac from DC Prototype 4 cubes. These cubes have a cap made with 30% w/w NaCl in PLGA.**

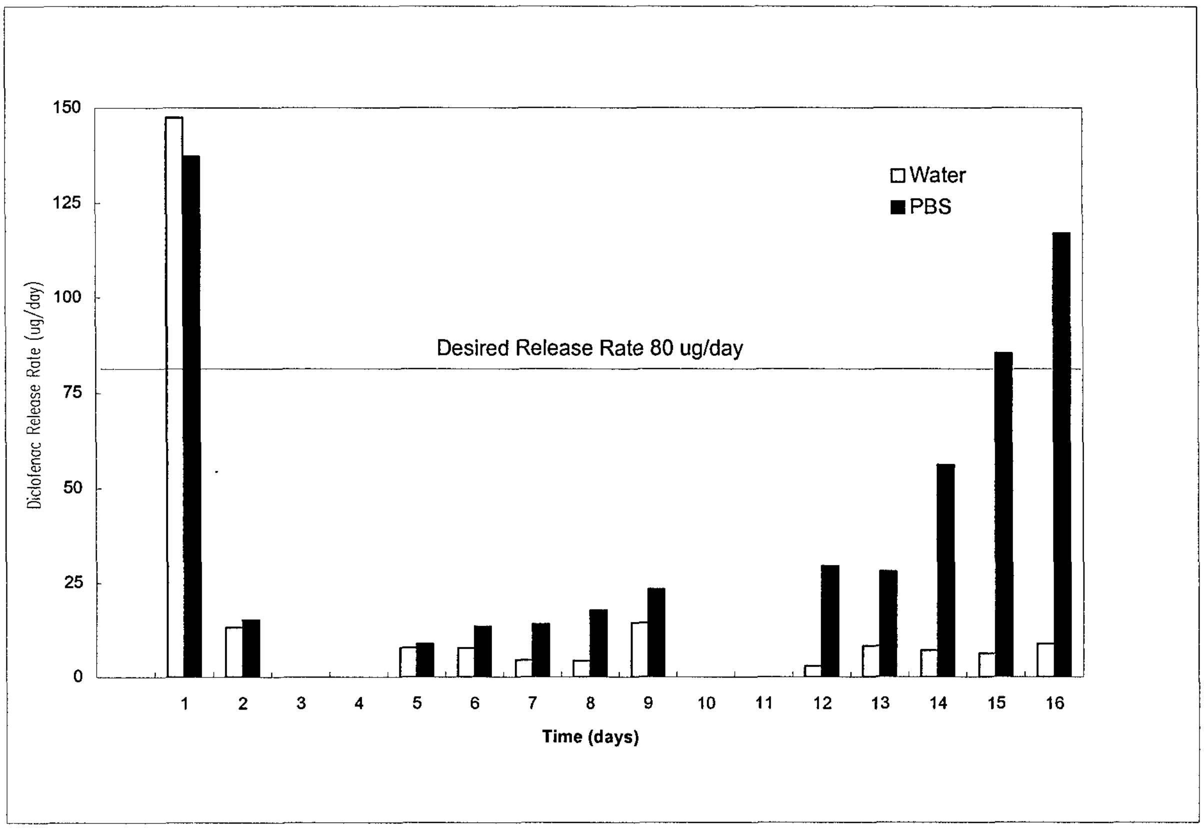


Figure 33. Diclofenac release rate from DC Prototype 4 cubes.

Table 8. Dimensions of Prototype 5 Cubes

Prototype	Weight (mg)	Height (mm)	Length (mm)	Width (mm)
5a	64 ± 1	2.3 ± 0.04	5.7 ± 0.1	5.6 ± 0.1
5b	80 ± 3	2.7 ± 0.1	5.5 ± 0.1	5.1 ± 0.1
5c	84 ± 2	3.0 ± 0.1	5.8 ± 0.1	5.6 ± 0.1

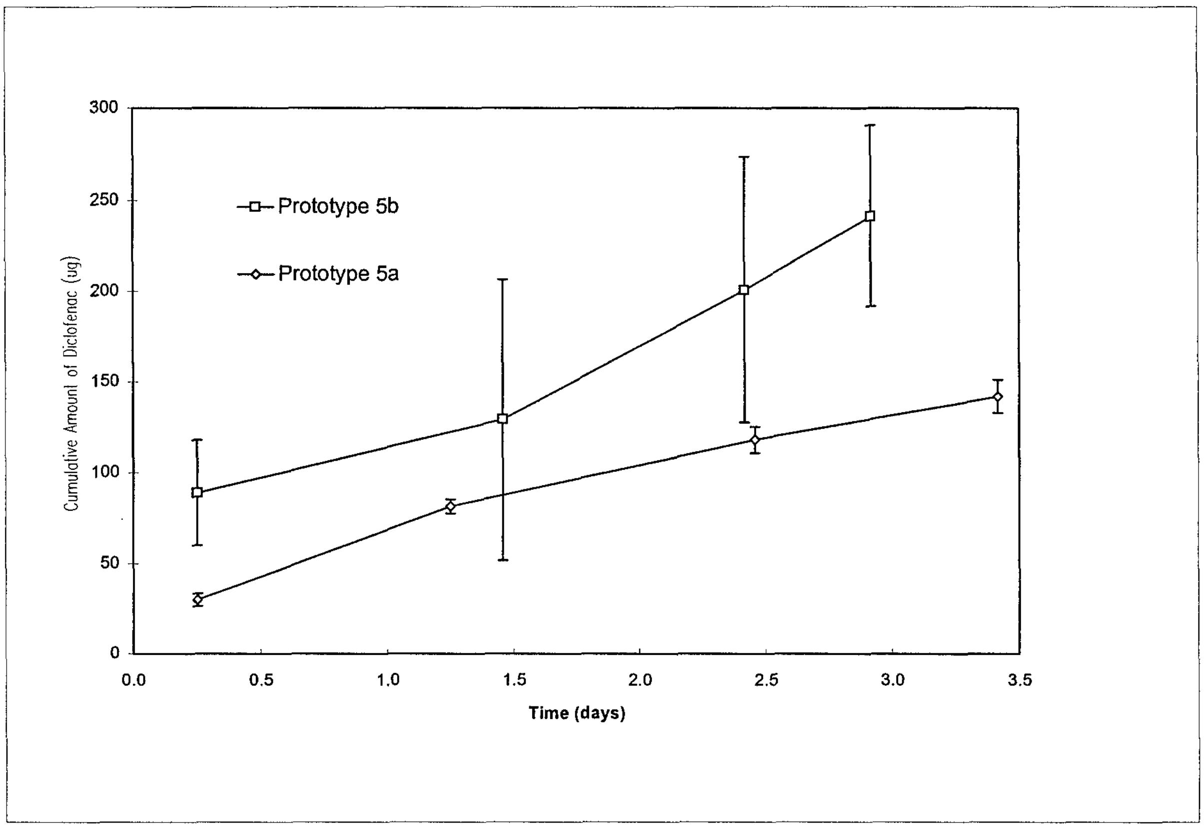


Figure 34. **Cumulative diclofenac release from DC Prototype 5 cubes.**

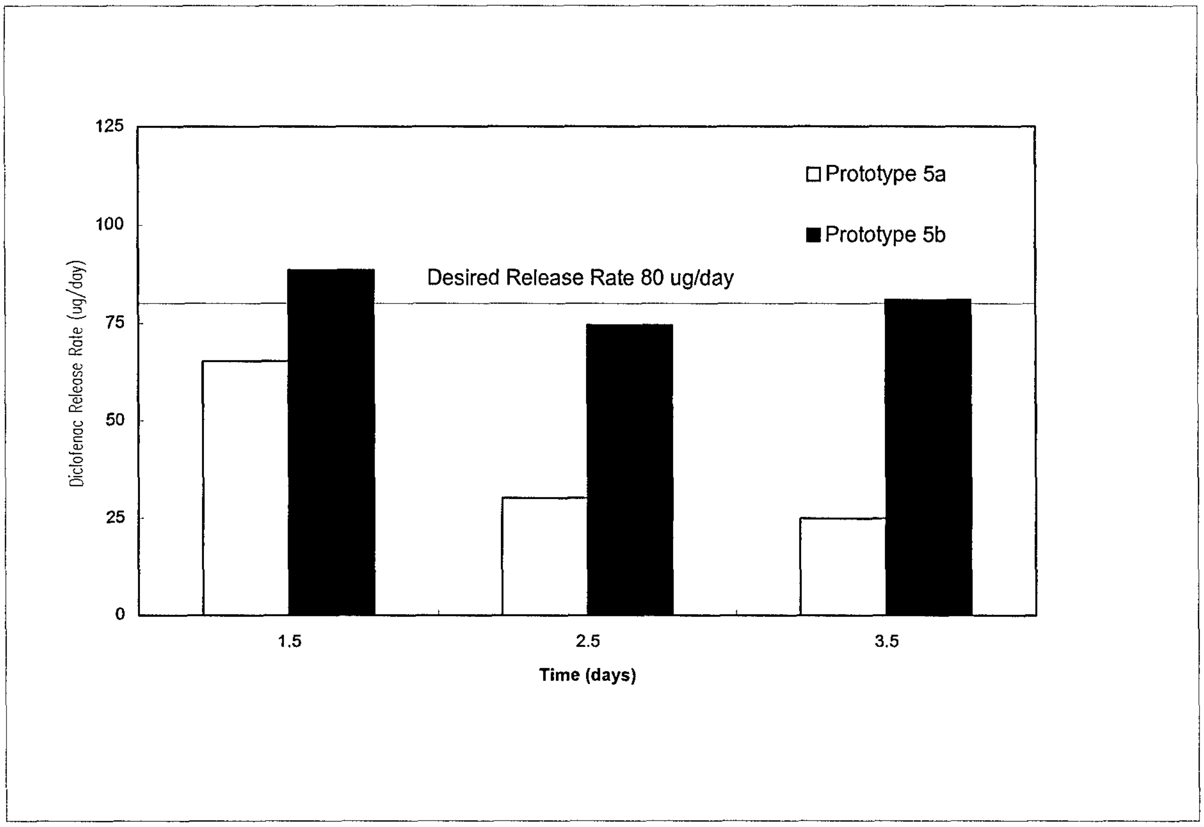


Figure 35. **Rate of diclofenac release from DC Prototype 5 cubes. This study was still in progress at the time this report was written. However, the initial data from Prototype 5b exhibits the desired drug release.**

2.3.2.4. Residual solvent removal

Before the implants can be used for animal testing or human use, it may be necessary to ensure that the residual solvent content is within acceptable limits. Based on prior experience, it is known that the residual solvent content in TheriForm™ fabricated implants can be significantly reduced by subjecting the fabricated implant devices to liquid CO_2 drying. However, exposure of PLGA (75:25) 60 KDa to liquid CO_2 caused shape distortion and considerable hardening. This can be attributed to the low glass transition temperature of this particular polymer. Thus, another criterion for polymer selection of material may be that the fabricated implant should be able to withstand drying, without compromising the structural and mechanical integrity of the implant as well as its drug content and drug release characteristics. Although some polymer systems may have perfectly ideal properties for thin film formation and degradation rates, they may have to be eliminated based on the inability to remove chloroform without distorting the overall shape and microstructure of the devices. For example, analysis indicates that implant components made with PLGA(75:25) 60 KDa may not be suitable for residual solvent removal by liquid CO_2 drying.

The amount of diclofenac in prototype 1 disks did not change upon exposure to liquid CO_2; thus, the exposure CO_2 for 5 minutes did not extract the active agent, see Table 9. The amount of residual chloroform in prototype 1 disks exposed to liquid CO_2 was $1.9 \pm 0.3\%$ ($n = 3$), compared to $5.5 \pm 0.3\%$ ($n = 3$) for control devices not exposed to liquid CO_2. Thus, exposure to liquid CO_2 for 5 minutes reduced the amount of chloroform by 65%. The prototype 3 devices whether exposed to liquid CO_2 for 30 minutes or 60 minutes had a 17% decrease in content. However, prototype 4 devices exposed to liquid CO_2 for 30 or 60 minutes did not have a change in diclofenac content from control devices. Thus, the increase in exposure time did not conclusively affect diclofenac content, rather the extraction of diclofenac by liquid CO_2 appeared to be dependent on the prototype batch. The increase in exposure time from 5 minutes to 30 minutes significantly improved the efficiency of removing residual chloroform; although, increasing the exposure time from 30 minutes to 60 minutes did not affect the residual chloroform content.

Table 9. Effect of liquid CO_2 exposure time on diclofenac content

Batch	CO_2 Exposure (min)	Diclofenac Amount (mg)	Control Diclofenac (mg)	Residual Chloroform (wt %)
Prototype 1	5	7.30	7.3 ± 0.2	2.19
Prototype 1	5	7.36	7.3 ± 0.2	1.67
Prototype 1	5	7.07	7.3 ± 0.2	1.83
Prototype 3	30	1.014	1.23 ± 0.02	0.35
Prototype 3	60	1.038	1.23 ± 0.02	0.39
Prototype 3	60	1.007	1.23 ± 0.02	0.17
Prototype 4	30	0.994	1.02 ± 0.09	0.12
Prototype 4	60	0.995	1.02 ± 0.09	0.18

2.3.3. Conclusions

Choosing an appropriate binder was a balance between being able to dissolve the polymer and dissolving the maximum amount of diclofenac. The best binder composition was determined to be 24 mg/ml of diclofenac in 1:4 methanol:chloroform.

Early diclofenac prototypes exhibited an initial burst of diclofenac release. This was corrected by coating the large face of the device with placebo polymer. In addition, the desired release rate of therapeutic quantity was achieved by mixing the diclofenac with inert NaCl in order to achieve the proper loading. This diclofenac-NaCl mixture formed an interconnected-phase within the polymer matrix. Prototype 5b was successful in achieving the 80 μg/day desired release rate for the time period tested to date.

Exposure of the devices to liquid CO_2 for 30 minutes significantly reduces the amount of residual chloroform. More studies are needed to determine if this process affects the concentration of diclofenac in the devices, but initial results are promising.

Note: Data on fabrication and dimensions of diclofenac implant prototypes referred to in this section can be found in Therics' laboratory notebook 001, pages 11-14, and 16, and notebook 018, pages 1-37. The data on the liquid CO_2 drying can be found in notebook 013 , pages 1-2, and 8-11.

3. SUMMARY OF ACCOMPLISHMENTS

The objective of this research was to investigate the feasibility of developing an intravitreal implant capable of pulsatile release of 5-FU, an anti-proliferative agent, while also concurrently releasing diclofenac, a non-steroidal anti-inflammatory drug, at a constant rate for the treatment of traction retinal detachment. All initial prototypes contained either only 5-FU or diclofenac in order to simplify the development process. However, all prototype implants contained a placebo structure to mimic the portion that would contain the other drug. Thus, the capability of TheriForm™ technology to fabricate devices that contain both drugs in the same device, but in separate sections, has already been demonstrated. The intention was to separately optimize the composition and architecture of the sections containing the two drugs and then to fabricate the preferred prototypes of each of the two drugs into a single, multi-drug implant. The six month Phase I study has yielded several positive results which are listed below:

Fabrication and testing of 5-FU implant

1. Implant architecture for pulsatile delivery of 5-FU was finalized. The design comprised of 4 chambers, each covered with a different lid. The lids differ in composition and/or thickness so as to vary in degradation rates and, thereby, impart pulsatile release of 5-FU from the chambers.

2. To facilitate selection of the appropriate material for fabricating different parts of the implant, thin films of several biodegradable polymers were made using the technique of solvent-casting and subjected to *in vitro* degradation studies in static phosphate buffered saline solution at 37°C to mimic the conditions of vitreous humor. Based on the results, specific PLGA and P(FAD-SA) polymers were selected for prototype fabrication.

3. Several 5-FU-containing prototypes were successfully fabricated using TheriForm™ technology which were tested for drug release in static phosphate buffered saline solution at 37°C. The quantitative analysis of 5-FU was performed using HPLC assays which were tested for linearity, precision, specificity, and sensitivity.

4. 5-FU release from uncovered chambers was rapid (within 3 hours) and complete, indicating that it is not a rate limiting step and that immediate drug release from individual chambers can be expected once their respective lids disintegrate.

5. 5-FU release from chambers fabricated with different composition and thickness of polyanhydrides (primarily P(FAD:SA)), exhibited different lag times raging from 0-18 hours. The immediate releasing lids are suitable for providing the first pulsatile release. However, the desired subsequent inter-pulse lag times are in the order of days and not hours. According to the results of the film degradation studies, the selected polymer materials should have exhibited longer lag times. The reasons for these observations were identified

following scanning electron microscopy of the implant prototypes. The primary reason was inadequate binding of the lids and the side walls of the chambers leading to delamination effects and premature leakage of the drug. This may be caused by the lack of adhesion between the materials comprising the lid and the walls or due to insufficient surface area on the top of the walls available for contact with the lid. Strategies to circumvent these problems are under investigation and will be utilized for product optimization during Phase II of this project.

Fabrication and testing of diclofenac implant

1. Several diclofenac-containing prototypes were successfully fabricated using TheriForm™ technology and were tested for drug release in static phosphate buffered saline solution at 37°C. An HPLC assay was developed and tested for linearity, precision, specificity, and sensitivity prior to quantitative analysis of diclofenac.

2. Initial prototypes of the diclofenac intravitreal implant exhibited an initial burst release, attributed to the large, drug containing surface area of the implant exposed to the dissolution medium, followed by continuous delivery of diclofenac at sub-therapeutic rates.

3. The second generation of prototypes was fabricated with the a large fraction of the diclofenac-containing component capped with placebo polymer layers of different thickness. This resulted in a slow initial drug release that gradually exceeded the target rate after a few days. Also, complete drug release was not observed within the desired sixteen days.

4. The prototypes described above exhibited drug release profiles at both extremes of the desired constant release rate of 80 μg/day. To increase the inter-connectivity of the diclofenac particles in the polymer phase without increasing drug loading, sodium chloride was added as an inert filler. Thus, subsequent were fabricated with a blend of PLGA polymer with sodium chloride. The addition of 35% (w/w) sodium chloride ensured that the combined loading of diclofenac and sodium chloride (w/w with respect to the polymer) was above the minimum necessary to create interconnected particles, as predicted by the percolation theory. These channel facilitated continuous and complete release of diclofenac from the implant. These prototypes were also covered with a placebo polymer coating to inhibit initial dose dumping by the implant. The initial results of this ongoing study indicate that one of the prototypes exhibits the target diclofenac release rate of 80 μg/day.

5. Preliminary experiments on post-fabrication exposure of the diclofenac prototypes to liquid CO_2 indicate that the procedure reduces the amount of residual solvent in the devices without affecting drug content. This method will be explored in more detail for residual solvent removal during Phase II studies.

Overall, the research conducted so far has not only demonstrated the capability of TheriForm™ technology to fabricate intravitreal implants capable of prescriptive drug release, but has provided a significant amount of fundamental information applicable to development of biodegradable implants in general. The data from film degradation studies can be utilized for constructing implants, or their components, with different degradation rates. Although the purpose of this ongoing research is to develop a device with specific drug release rates, the prototype designs can serve as a platform for other multi-drug applications.

4. SIGNIFICANCE OF RESEARCH

This study clearly demonstrates the unique capability of TheriForm™ technology to fabricate biodegradable implants for the following drug release profiles:

1. Pulsatile drug release, as demonstrated using 5-FU.

2. Continuous drug release, as shown using diclofenac.

3. Prescriptive release of two or more drugs located in separate, architecturally different compartments within the same device. Although in this study an implant containing both 5-FU and diclofenac was not fabricated, placebo or single drug-containing prototypes similar to the design illustrated in Figure 1 were successfully constructed. In addition, most single-drug prototypes subjected to release studies contained a placebo structure that mimicked the portion that would contain the other drug in the commercial dosage form. Fabrication of such multi-drug devices, normally unachievable by conventional techniques, can be easily accomplished by TheriForm™ process.

Thus, this research provides more than sufficient evidence that TheriForm™ technology has the potential to develop biodegradable implants capable of prescriptive drug release of more than one drug for the treatment of ocular diseases and injuries. In addition, significant fundamental knowledge about biodegradable implants in general has been acquired during this project that can serve as a platform for development of several implants for combating several diseases, such as:

1. Delivery of drugs for treatment of other ocular diseases such as retinal tumors, diabetic retinopathy, age related macular degeneration and cytomegalovirus retinitis.

2. Implants for other diseases such as tuberculosis, cancer, and bone defects.

3. Implants for vaccines.

4. Subcutaneous systems for hormone replacement therapy and drug abuse.

The treatment of these and other conditions is anticipated to be more successful utilizing TheriForm™ technology to fabricate implants than any presently known conventional approach. Most available technologies are limited by the machines or tools used in fabrication. On the other hand, TheriForm™ technology is an additive process, where microscopic features are incorporated layer by layer. The ability to control both the microscopic features as well as the macroscopic shape simultaneously with TheriForm™ is being exploited to design and fabricate several unique polymeric drug delivery systems. The mode of release can also be tailored so that drugs will release at a constant rate, in pulses, or a combination of both within one TheriForm™

delivery system. Design and fabrication of resorbable and non-resorbable polymeric drug delivery systems are major foci of Therics' TheriForm™ technology development activities.

5. FUTURE WORK: PHASE II PLANS

This research has been the first step for Therics towards completion of a technical investigation of applying the TheriForm™ technology for fabrication of prescriptive controlled drug delivery systems for retinal therapy. It is anticipated that Therics will take a TheriForm™ retinal drug delivery system product line to the marketplace, possibly as an integral part of a future ophthalmic franchise. To reach this goal, the following issues need to be addressed during the next phase of the project:

1. Optimize structure and composition of the implant and confirm 5-FU and diclofenac dose to achieve desired drug release profiles.

2. Decrease size of the implant and improve design to eliminate sharp corners and edges.

3. Combine the two sub-structures for 5-FU and diclofenac into a single multi-drug device capable of providing independent, prescriptive release rates.

4. Design an anchor onto the device so that it may be sutured in the inner walls of the vitreous cavity during surgical placement inside the eye.

5. Reduce residual solvents without affecting drug content and release rate.

6. Explore alternative mechanisms for automated binder and drug solution deposition, particularly for deposition of 5-FU at desired locations inside the implant. Therics' Engineering department is in the process of constructing a new mechanism for binder deposition that will significantly enhance TheriForm™ process capabilities.

7. Investigate methods for fabrication of sterile implantable products. This will include aseptic manufacturing and/or post production sterilization.

8. Perform stability tests on the implants to assess packaging requirements, storage conditions, and shelf life.

9. Evaluate *in vivo* drug release profile of TheriForm™ implants and suitable control systems in an appropriate animal model, such as the rabbit.

10. Conduct a market survey for commercialization of the intravitreal implants for treatment of traction retinal detachment and other ocular diseases.

If these studies are successful in demonstrating superior performance to traditional devices, Therics will be uniquely positioned to begin human clinical trials in concert with a strategic commercial partner.

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7. APPENDICES

7.1. APPENDIX A

TheriForm™ (Three Dimensional Printing Technology)

TheriForm™ is a fabrication process which allows control over both structure and composition of implantable drug delivery systems. This is achieved at three levels: (1) macroscopic shapes, (at the cm level); (2) intermediate features, such as size, orientation and surface chemistry of pores and channels, (at the ~100 μm level); and (3) microscopic features, including porosity in the structural walls of the device, (at the ~10μm level). TheriForm™ is a solid free-form fabrication technique, in which objects are built in a laminated fashion through sequential addition of patterned thin layers. The information needed to form these two dimensional segments is obtained by calculating the intersection of a series of planes with the computer aided design (CAD) rendition of the object. A schematic for a typical TheriForm™ process is shown in Figure 36.

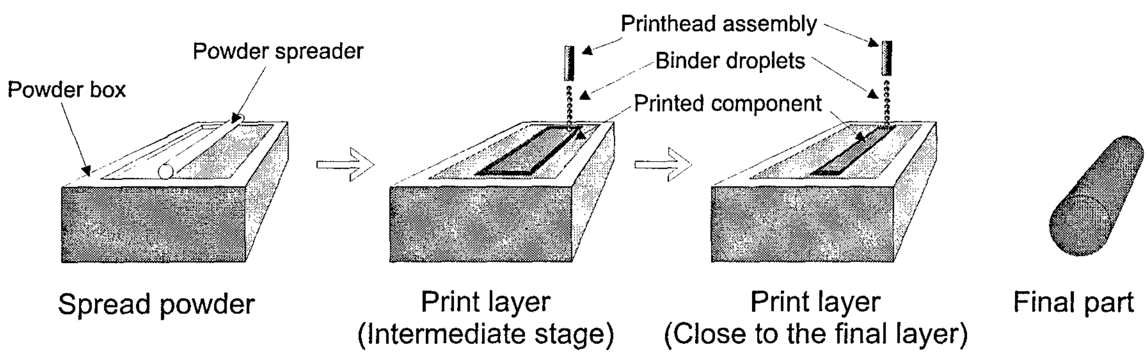

Figure 36. Schematic of TheriForm™ Process.

A thin layer of a powder is spread in a box, then the print-head assembly scans over the powder bed depositing binder droplets onto selected regions. TheriForm™ employs ink-jet printing technology to generate and place binder droplets. In regions where the binder is printed, the binder holds the powder together through a variety of different and material-specific interactions, thus creating the solid regions within the 2-dimensional slice. The floor of the power box drops down , and a new layer of powder is spread. Information for the next layer is relayed from computer and then printed. The process of spreading powder and printing is repeated until construction of the object is complete. A more detailed explanation of the TheriForm™ technology and its applications in drug delivery systems and the fabrication of dense polymeric structures has recently been described.[15-19]

A broad spectrum of materials can be used in the TheriForm process. Virtually any material which can be made into a powder and bound with a liquid is a candidate for a matrix material for this fabrication technique. Components have already been constructed from metals, ceramics, polymers, and hydrogels. In addition, different materials can be dispensed through separate nozzles, which is a concept analogous to color ink jet printing. Materials can be deposited as particulate matter in a liquid vehicle, as dissolved matter in a liquid carrier, or as molten matter. The proper placement of droplets can be used to control the local composition and to fabricate components with true three dimensional composition gradients.

The ability to control both the macroscopic shape and microscopic features simultaneously with TheriForm has been exploited to design and fabricate polymeric drug delivery systems.[16] CAD software was used to design an implantable drug delivery system which releases multiple drugs in a prescribed fashion. The mode of release can also be tailored so that drugs will release at a constant rate, in pulses, or a combination of both within one TheriForm delivery system. Design and fabrication of resorbable and non-resorbable polymeric drug delivery systems are major foci of Therics' TheriForm technology development activities.

7.2. APPENDIX B

Glossary of terms and abbreviations

Binder	Any of the materials that are used in liquid form and dispensed from the nozzle on the TheriForm™ machine
DC	Diclofenac sodium, an anti-inflammatory drug
5-FU	5-Fluorouracil, an anti-proliferative agent
Flow Rate	Amount of binder dispensed from the nozzle in a given time.
Layer Thickness	The increment that the piston is dropped to allow powder spreading during TheriForm™ processing.
Line Spacing	Displacement between adjacent lines in a TheriForm™ product. This is the distance the slow axis is incremented between passes of the fast axis during TheriForm™ processing.
Mask	A brass or steel sheet with cutouts that is placed over the powder bed during TheriForm™ processing to create the overall shape of a TheriForm™ product. Also known as a stencil.
Powder	Any of the materials that are used in solid form in the bed of the TheriForm™ machine.
Print Speed	Speed of the binder dispensing module (or print-head) on the fast axis of the TheriForm™ machine. Often this speed is expressed as a percentage of maximum speed. The maximum speed of the MIT Hood Machine is 150 cm/s and the maximum speed of the Alpha-0 machine in Princeton is 200 cm/s.
Solvent Removal	Post-processing steps to reduce levels of processing solvents (usually chloroform) in TheriForm™ products. The current process for solvent removal is liquid or supercritical CO_2 extraction.
Stencil	see Mask.
Template	see Mask

7.3. APPENDIX C

Gantt chart of performance schedule

Proposed Performance Schedule x indicates scheduled tasks

Tasks	Week #																									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1. Choice of drugs	x	x																								
2. Choice of polymer matrix	x	x																								
3. Fabrication of thin sheets			x	x	x	x	x	x	x	x																
4. Diffusion cell studies									x	x	x	x														
5. Characterization of sheets									x	x	x	x														
6. Prototype system design	x	x											x	x												
7. TheriForm prototypes															x	x	x	x	x	x						
8. Prototype physical testing																		x	x	x	x			x	x	
9. Assay of drugs & polymer matrix																		x	x	x	x	x	x			
10. Final report writing																								x	x	x

Actual Progress + indicates weeks during which tasks were performed

Tasks	Week #																									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1. Choice of drugs	+	+																								
2. Choice of polymer matrix	+	+	+	+	+	+																				
3. Fabrication of thin sheets					+	+	+	+	+	+	+	+	+	+	+	+	+	+								
4. Diffusion cell studies	Diffusion studies were not performed.																									
5. Characterization of sheets											+	+	+	+	+	+	+	+	+	+						
6. Prototype system design	+	+															+	+	+	+	+	+		+	+	
7. TheriForm prototypes												+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8. Prototype physical testing													+						+	+	+	+	+	+	+	+
9. Assay of drugs & polymer matrix									+						+	+	+	+	+	+	+	+	+	+	+	+
10. Final report writing																									+	+

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 4 Dec 02

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Phylis Rinehart

Encl

PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management

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